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Erratum to Vol. III.

Page 100, line 5, *for* '+0·85±0·045' *read* '−0·85±0·045'

NOTICE

The Indian Journal of Agricultural Science

A Bi-monthly Scientific Journal of Agriculture and the Allied Sciences, mainly devoted to the publication of the results of original research and field experiments.

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ORIGINAL ARTICLES

STUDIES IN INDIAN PULSES.

(3) THE TYPES OF *CAJANUS INDICUS* SPRENG.

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(With Plates I-IV and one text-figure)

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I. Introduction.

The pigeon pea, *Cajanus indicus* Spreng., is one of the most widely cultivated pulses in India. The plant is a leguminous shrub and although it has long been a cultivated crop in India, it is doubtful if it occurs wild anywhere in this country. It has been reported to occur in the wild state in Africa in the region of the Upper Nile and in the coast districts of Angola. The plant appears to have been introduced into the West Indies by the slave trade and has now been taken to Brazil, Guiana and most of the warm parts of the American continent. It was introduced into Australia about fifty years ago and grows well in the warmer parts of the Commonwealth being recommended [Turner, 1892] as a fodder crop, and also as a

vegetable, for cultivation in the north-eastern area of New South Wales. If the view that the species is endemic in Africa be accepted, it is probable that the plant reached India by ancient traders trading on the route between Zanzibar, India and Ceylon.

Hooker [1879] classifies the genus *Cajanus* into a single species *Cajanus indicus* Spreng., but some authors have described two distinct species, *C. flavus* and *C. bicolor*, the former having a yellow standard and the latter having a yellow standard streaked with purple. According to Duthie and Fuller [1883] the form *C. flavus* is known under the name "*tur*" and is commonly cultivated in the Central Provinces, while *C. bicolor* is the "*arhar*" of the United Provinces. Roxburgh [1832] names the plant *Cytisus Cajan* Willd., and states that the flowers are yellow—his diagnosis is not very critical. Other differences between the two forms are that *C. flavus* is described as a relatively small plant having only two to three seeds in a pod which is more spotted while *C. bicolor* is a larger plant with four to five seeds in the pods which are marked with dark streaks. The value of these characters will become apparent when we consider the classification of the 86 types which have been isolated at Pusa.

The present research arose out of the investigation on the isolation of a wilt-resistant type of *rahar*. This work is the subject of another publication [McRae and Shaw, 1933] and while it is unlikely that a higher degree of resistance to disease than that which is present in the Type 80 (A_2) will be obtained in any other type of *rahar* which is described in this paper, yet it is obviously desirable that all the possible pure lines in the crop should be tested for their reaction to the disease. This should afford some insight into the possible correlation of morphological characters with the physiological property of resistance and at the same time may give a choice of resistant types which differ in their agricultural properties.

It is impossible to make any reliable estimate of the extent to which *Cajanus indicus* is cultivated in India. In official statistics it is placed under pulses and other food grains of which the total area in India in recent years has been approximately 30 million acres. The crop is more extensively cultivated in Bihar, north Bengal and the eastern part of the United Provinces than in the more northerly tracts of India, the susceptibility of the plant to damage from frost limiting its cultivation in Northern India. It is frequently grown as a mixed crop with maize (*Zea Mays*) or *juar* (*Andropogon Sorghum*) and as a rotation crop for cereals. When grown as a mixed crop with maize yields of 500 to 1,500 lb. per acre can be obtained; when grown as a pure crop the yields are approximately 800 to 1,800 lb. per acre. The different types described in this paper vary considerably in yielding power and are being tested for this character. In Bihar and the United

Provinces of Agra and Oudh the crop is normally sown in July with the first rains of the monsoon and ripens about March; it thus occupies the land during one rainy season and one cold weather. The plant is hardy and requires little attention in the field; the crop, however, benefits greatly from inter-cultivation at the beginning of the cold weather and for this reason the erect types of *rahar* which allow of the easy passage of bullocks and implements between the rows of plants are to be preferred to those with a bushy and straggling habit. Sowing is normally done in rows 2½ to 3 feet apart.

In India the grain is highly esteemed as a food both for man and cattle. The plant owes, however, a large measure of its popularity to the fact that it is a leguminous crop and consequently possesses valuable properties as a restorative of nitrogen to the soil. The roots also aerate the soil and sub-soil, and the vegetative parts add a lot of organic material to the soil by the enormous leaf and flower shedding. It is this property which renders it an economic success, as otherwise the return from the crop is not commensurate with the length of time for which it occupies the ground.

II. Pollination.

The biology of the plant in the Central Provinces has already been adequately dealt with [Mahta and Dave, 1931] and it must be remembered that the observations in this paper apply to the conditions in Bihar.

Flowering extends over a very long period. In local Bihar varieties the first flowers generally appear at the end of November or early in December and flowers are still being produced in March when the bulk of the crop is ripe. Self-pollination, as in many other plants of this order, is the rule, but crossing takes place freely, the plants having many insect visitors. Seed-setting is very largely dependent on the weather and is favoured by bright sunny days. Damp and cloudy weather has a very adverse effect on seed-setting and the yield of seed in Bihar is greatly reduced if dull weather prevails in January and February. An estimate of the amount of natural crossing was made by Howard and others [1920], who state that the proportion of heterozygotes in the progeny from unbagged plants of a definite pure line was 2·25 per cent. when the estimation of heterozygotes was based on the characters of the flower. If the characters of the seed are also considered the proportion rises to 5-6 per cent. Further observations in the seasons 1927-28 and 1929-30 made on ten different cultures grown on a large scale showed that the percentage of natural crossing based on observations on flower colour, pod colour and plant habit ranged from 0·15 to 7·59 per cent. The estimation of the proportion of heterozygotes depends upon the ease with which the heterozygote can be distinguished from the type of the parent culture. This was most readily done in Type 16 in which the back of the

standard is pure yellow without any red marks and in which this type of standard is recessive to forms having red marks on the standards.

Percentages of natural crossing in rahar *types.*

Type	1927-28		1929-30	
	Percentage of crossing	Number of plants examined	Percentage of crossing	Number of plants examined
4	2·6	6,070	7·59	580
5	0·14	3,600	0·59	851
16	6·7	2,238	6·73	1,427
25	0·99	10249	..	..
59	1·5	4,720	1·66	1,085
51	2·9	3,980	6·83	600
72	0·09	5,430	..	..
73	0·32	18,093	2·07	770
69	0·56	8,473	1·04	1,723
82	0·16	5,618	0·63	831

This experiment was carried with unbagged seed which had been obtained in 1926-27 from cultures growing in lines among other cultures. The conditions were, therefore, exceptionally favourable for cross-fertilization. Much higher percentages of crossing have, however, been recorded in the Central Provinces.

III. The morphological characters.

(*1*) *Height.*—The height of types of *rahar* grown in Pusa ranges from 180 cm. up to 375 cm. In this classification plants up to a height of 225 cm. have been classed as short, between 225 and 325 cm. as medium in height, and above 325 cm. as tall.

(*2*) *Habit.*—The habit varies from a straggling bushy growth to an erect habit (Plate I) resembling that of the poplar tree, in which there is a main axis and the laterals grow in a vertical direction. The angle which the secondary branches make with the main stem can be measured and affords an easy method of distinguishing the different habits. Since the branches are generally curved at the base with the concave side towards the main stem, the actual angle at the axil does not give a reliable criterion of the habit and it is better to measure the angle made by a line joining a point at a certain definite height on the branch with the main axis at a point of origin of the branch (Fig. 1). The definite point on the branch which was chosen for this measurement was situated at a vertical height of 15 cm. above the horizontal plane through the axil of the branch. The measurement of this angle can be made very easily by means of an L-shaped iron bar of which the short limb is 15 cm. long, and the long limb is graduated in centimetres and millimetres. The size of the angle made by joining any point on the long limb to the end of the short limb can then be determined directly from a table of tangents. In using this

instrument the short limb is placed in the axil of the branch as in Fig. 1, and the point at which the branch crosses the long limb is read off on the scale. In actual practice it is convenient to graduate the long limb directly in angular differences and this was done in our work.

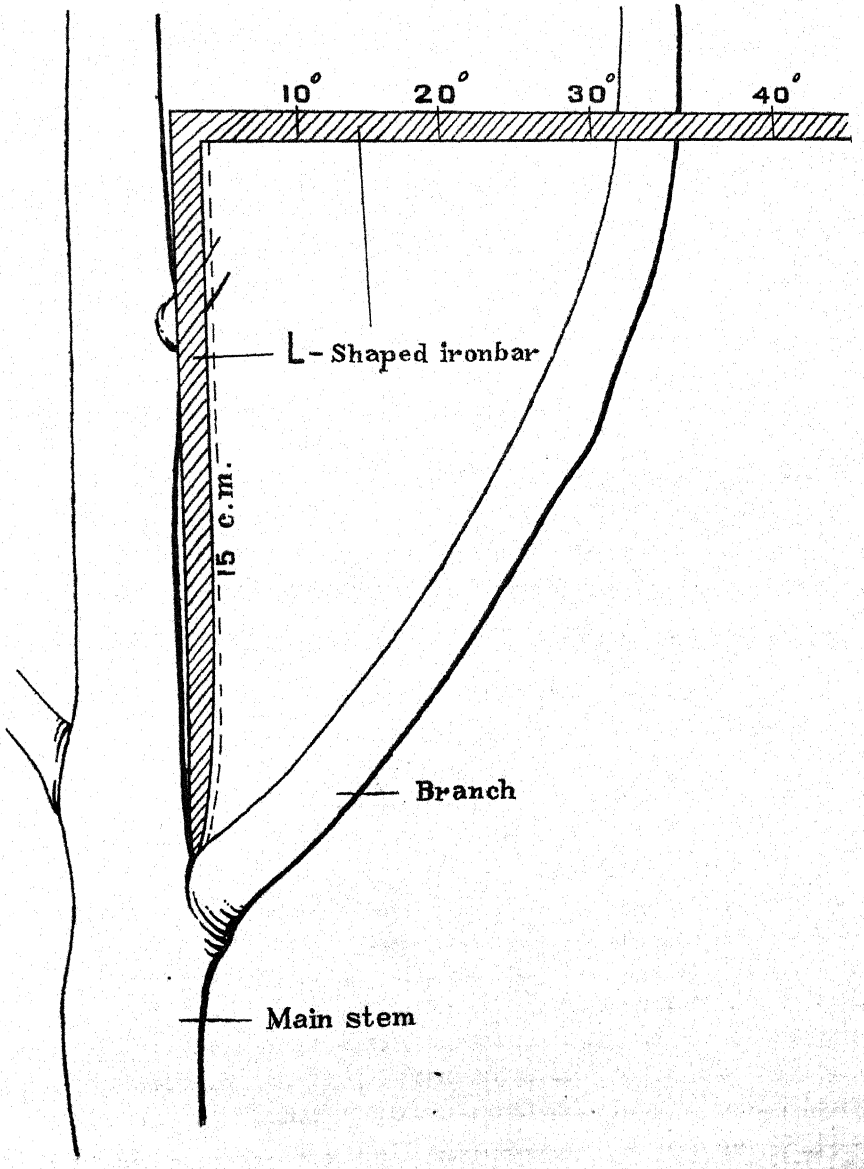

Fig. 1

In erect types of *rahar* the average angle made by the branches with the main stem is from 20° to 35° while in spreading types this angle varies from 40° to 55°. The erect and spreading habits can also be distinguished by the ratio of greatest breadth to height of the plant, *i.e.*, $\frac{\text{Greatest breadth}}{\text{Height}}$. This is about 0·3 to 0·4 in erect types and in spreading plants may reach up to 0·8, but it varies with the spacing of the plants.

Spreading plants may be divided into two classes according to the character of the branching. In one the branches are straggling and the flowering portion of the branch is long, unbranched and conspicuous, with few secondary and tertiary branches (Plate I, Type 62). In the other class the number of secondary and tertiary branches is much greater and the flowers are not distributed over such a length of the branch (Plate I, Type 80). These two habits are termed straggling and spreading respectively.

Erect plants can also be divided into two classes according to the types of the inflorescence.

According to habit, therefore, we may distinguish :—

(1) Plants erect with inflorescence crowded (Plate I, Type 5).

(2) Plants erect with inflorescence scattered.

(3) Plants spreading with straggling branches (Plate I, Type 62).

(4) Plants spreading with branches not straggling (Plate I, Type 80).

(3) *Stem colour and leaf-vein.*—All the types when young develop some red or purple colour on the stem and branches exposed to sunlight, which fades away after some time but appears again when the plants are old. The amount of colour varies in different types and in different individuals of the same type to such an extent that we have not used this as a diagnostic character.

In some types leaf-veins are green and develop no colour but in some they develop reddish purple colour mainly on the midrib. The amount of purple colour varies in different types and we have not attempted to use this character in the classification of the types.

(4) *Maturity.*—Early types flower in Bihar from the first week of October till the third week of November and in these flowering is practically over by the end of January but a second flush of flowers often occurs. These types do not yield well in Bihar. The local Bihar varieties begin to flower during November and continue flowering up to the end of February or even until harvest. The long flowering season in *rahar* is a matter of inconvenience as the first formed pods split and shed their seed before harvest. The time of harvest is, therefore, a matter of judgment depending on the ratio of the amount of grain shedding to the number of late-formed pods.

(*5*) *Flower colour.*—The petals are yellow and flowers are classified according to the depth of this yellow and the degree and distribution of red colour on the standard. The red colour may be present in the veins or diffused on the dorsal side of the standard, it may form a complete covering to the back of the standard, masking the yellow colour. The red veins generally radiate from the base of the standard. Red colour may also be present as small dots on the margin of the dorsal side of the standard; to this condition we have given the name "ringed". The following types of flower are distinguished (Plate II):—

I. Dorsal side of standard yellow with few or no red marks at the base.
  1. Ventral side of standard very pale yellow, Types 1, 2, 3.
  2. Ventral side of standard pale yellow.
    (*a*) Dorsal side margin with faint red dots, *i.e.*, "ringed", Type 4.
    (*b*) Dorsal side of standard not ringed, Types 5-10.
  3. Ventral side of standard deep yellow, Types 11-25.

II. Dorsal side of standard yellow with red veins radiating from base of standard.
  A. Very slight or no diffused red colour at the base of dorsal side of standard.
    1. Ventral side of standard pale yellow, Types 26-28.
    2. Ventral side of standard deep yellow, Types 29-65.
  B. Red colour present as a diffused half ring at the base of dorsal side of standard.
    1. Ventral side of standard pale yellow, Types 66, 67.
    2. Ventral side of standard deep yellow, Types 68-71.
    3. Ventral side of standard orange, Types 72-74.
  C. Red colour diffused on dorsal side of standard and red veins distinct.
    1. Ventral side of standard pale yellow, Type 75.
    2. Ventral side of standard orange, Types 76-79.

III. Red colour diffused on dorsal side of standard, red veins generally not visible.
  A. Red colour existing in patches, Types 80-82.
  B. Red colour uniformly covering dorsal side of standard.
    (*a*) Dorsal side of standard light red, Type 83.
    (*b*) Dorsal side of standard deep crimson.
      1. Ventral side of standard pale yellow, Types 84, 85.
      2. Ventral side of standard deep yellow, Type 86.

(*6*) *Inflorescence.*—The inflorescence may be open or closed. In the open type flowers are borne at fairly long internodes and in the closed types the flowering

branches arise close together at the end of a branch. This latter type is described as "crowded" in contrast with the "open" or scattered type. The inflorescence in both cases is a raceme.

(7) *Pods.*—The pods vary in size, colour and shape. The length ranges from 4·4 to 8·5 cm.; pods from 4 to 5·8 cm. are classed as short, from 5·8 to 7·4 cm. as medium and above 7·4 cm. as long. The breadth of pods ranges from 0·6 to 1·4 cm., breadths below 0·85 cm. are called narrow, from 0·85 to 1·1 cm. medium, and above 1·1 cm. broad. Pods in the unripe stage are either green or green with black or red markings which may be so extensive as to cover the pod completely, masking the green colour. Pods are sometimes constricted between the seeds and such pods are called "beaded" in contrast with pods which are not constricted and which are termed not beaded. Pods are crowded or scattered according as the inflorescence is of the closed or open type.

The following classes of pods may be distinguished according to colour (Plate III):—

I. Pods green with or without a few black spots.

II. Pods green and red.

- A. Red colour in streaks.
- B. Red colour diffused over whole pod leaving green colour in streaks and patches.

III. Pods green and black.

- A. Black colour in streaks.
- B. Black colour diffused leaving green streaks.
- C. Black colour covering whole pod, no green visible.

(8) *Seed.*—The seed varies in shape, colour and size. Shape may be round or lens-shaped, colour ranges from white to deep purple and black.

The following types of seed have been distinguished (Plate IV):—

(1) Seed coat white with grey patch round the hilum, mass colour white, *e.g.*, Types 11, 29.

(2) Seed coat silver-white with numerous small faint grey marks; mass colour silver-white, *e.g.*, Types 13, 4, 30.

(3) Seed coat silver-white with numerous small faint brown dots; mass colour silver-white, *e.g.*, Type 7.

(4) Seed coat silver-white with heavy grey markings and some brown spots; mass colour silver-white with a brown tinge, *e.g.*, Types 1, 20, 26, 28, 31.

(5) Seed coat brownish fawn, *e.g.*, Types 3, 9, 82.

(6) Seed coat uniform greyish fawn, *e.g.*, Type 24.

(7) Seed coat light fawn background with brown markings; mass colour faint brown, *e.g.*, Types 8, 21, 36, 66.

(8) Seed coat uniform dark olive-grey, *e.g.*, Type 63.

(9) Seed coat dark olive-grey nearly covered with black markings, *e.g.*, Types 25, 65.

(10) Seed coat uniform light silver-fawn, *e.g.*, Type 35.

(11) Seed coat fawn with brown markings; mass colour light brown to yellowish brown, *e.g.*, Types 49, 56, 81, 22, 68.

(12) Seed coat brownish red, *e.g.*, Types 62, 10, 72.

(13) Seed coat silver-white with purple markings, *e.g.*, Types 73, 70, 78.

(14) Seed coat black, *e.g.*, Types 74, 79.

IV. KEY TO THE TYPES.

In classifying the types we have given first importance to the characters of flower, seed and pod. Characters such as habit, time of maturity and stature afford less reliable taxonomic criteria as they are to a certain extent influenced by the environment.

I. Flowers—dorsal side of the standard with a few or no red marks.

1. Ventral side of the standard very pale yellow.

A. Seed coat silver-white with fairly heavy brown spots, mass colour silver-white with faint brown tinge.

(a) Seed round . . . . . Type 1

(b) Seed lentil-shaped . . . . . Type 2

B. Seed coat brownish fawn . . . . . Type 3

2. Ventral side of the standard pale yellow.

A. Seed coat silver-white with faint grey markings, mass colour silver-white.

(a) Dorsal side of standard "ringed", *i.e.*, with red dots on margin, pods green . . . . Type 4

(b) Dorsal side of standard not ringed, pods green with black streaks.

(i) Pods crowded, plants erect . . . Type 5

(ii) Pods scattered, plant habit straggling . Type 6

B. Seed coat silver-white with numerous small faint brown dots, mass colour silver-white . . . Type 7

C. Seed coat with light fawn background, nearly covered with brown markings, mass colour faint brown . . . . . Type 8

D. Seed coat brownish fawn . . . . . Type 9

E. Seed coat brownish red . . . . . Type 10

3. Ventral side of the standard deep yellow.
   A. Seed coat white, with grey patch round the hilum, hilum brown, mass colour white.
      (a) Habit erect . . . . . Type 11
      (b) Habit spreading . . . . . Type 12
   B. Seed coat silver-white with numerous small faint grey marks, mass colour silver-white.
      (a) Pods green . . . . . Type 13
      (b) Pods green with black streaks
         (i) Pods crowded . . . . . Type 14
         (ii) Pods scattered
            (1) Plant habit erect
               †Seed small . . . . . Type 15
               ††Seed large . . . . . Type 16
            (2) Plant habit spreading
               (a) Pods beaded
                  †Plants medium in height . Type 17
                  ††Plants tall . . . . Type 18
               (b) Pods not beaded . . . Type 19
   C. Seed coat silver-white with heavier grey markings than Types 13-19 and with brown spots; mass colour silver-white with faint brown tinge . . Type 20
   D. Seed coat with light fawn background, nearly covered with brown markings, mass colour faint brown . . . . . Type 21
   E. Seed coat with fawn background having brown markings; mass colour brown.
      (a) Pods red with green streaks . . . . Type 22
      (b) Pods green with black streaks . . . Type 23
   F. Seed coat uniform greyish fawn . . . . Type 24
   G. Seed coat dark olive-grey, nearly covered with black markings . . . . . Type 25

II. Flowers—dorsal side of standard with red veins.
   A. Very slight or no diffused red colour at the base of dorsal side of standard.
      1. Ventral side of the standard pale yellow.
         (a) Seed coat silver-white with heavy grey markings and some brown spots; mass colour silver-white with faint brown tinge.
            (i) Pods medium in length and seed round . Type 26

(ii) Pods short and seed lentil-shaped.
(a) Seed medium . . . . . Type 27
(b) Seed small . . . . . Type 28

2. Ventral side of the standard deep yellow.
(a) Seed coat white with grey patch round the hilum; hilum brown; mass colour white . Type 29
(b) Seed coat silver-white with numerous small faint grey dots; mass colour silver-white . Type 30
(c) Seed coat white with heavier grey and brown spots than Type 30; mass colour silver-white with faint brown tinge.
(i) Pods green with black streaks.
†Plant habit straggling, pods beaded . Type 31
††Plant habit spreading, pods not beaded. Type 32
(ii) Pods black.
†Seed small . . . . . Type 33
††Seed medium . . . . . Type 34
d. Seed coat uniform light silver-fawn . . Type 35
e. Seed coat light fawn background with brown markings; mass colour fawn to faint brown.
(i) Pods green . . . . . Type 36
(ii) Pods red with green streaks . . . Type 37
(iii) Pods green with black streaks.
†Early in maturity.
*Pods not beaded . . . . Type 38
**Pods beaded . . . . . Type 39
††Late in maturity.
(a) Plants medium in height . . Type 40
(b) Plants tall . . . . . Type 41
(iv) Pods black with green streaks . . . Type 42
i. Seed coat brownish fawn.
I. Pods green with black streaks.
1. Plant habit straggling . . . Type 43
2. Plant habit spreading.
(a) Plants early in maturity, pods not beaded . . . . Type 44
(b) Plants late.
†Pods not beaded . . . Type 45
††Pods beaded . . . Type 46

II. Pods black.
(i) Plant habit erect, plants late in maturity, pods beaded . . . Type 47
(ii) Plant habit spreading, plants early in maturity, pods not beaded . . Type 48

(g) Seed coat with fawn background having brown markings, mass colour light brown to yellowish brown.
(i) Pods green . . . . . Type 49
(ii) Pods red with green streaks.
1. Seed medium . . . . . Type 50
2. Seed large . . . . . Type 51
(iii) Pods green with black streaks.
1. Plant habit erect . . . . Type 52
2. Plant habit spreading.
†Plants early in maturity . . . Type 53
††Plants late in maturity.
* Pods not beaded.
(a) Plants tall.
(i) Seed small . . . . Type 54
(ii) Seed medium . . . Type 55
(b) Plants medium in height.
(i) Seed small . . . . Type 56
(ii) Seed medium . . . Type 57
** Pods beaded . . . . Type 58
(iv) Pods black having green streaks.
1. Habit erect, late in maturity.
(a) Plants tall . . . . . Type 59
(b) Plants medium in height . . Type 60
2. Habit spreading, early in maturity . Type 61

(h) Seed coat brownish red . . . . Type 62

(j) Seed coat dark olive-grey.
(i) Pods green with black streaks . . Type 63
(ii) Pods black having green streaks . . Type 64

(k) Seed coat dark olive-grey nearly covered with black markings . . . . . Type 65

B. Red colour present as a diffused half ring at the base of dorsal side of standard.

1. Ventral side of standard pale yellow. Seed coat with light fawn background, nearly covered with brown markings; mass colour faint brown.

(i) Pods not beaded . . . . . Type 66
(ii) Pods beaded . . . . . Type 67

2. Ventral side of standard deep yellow.

(i) Seed coat fawn with brown markings; mass colour light brown.

† Pods green, medium in size, seed lentil-shaped . . . . . Type 68

†† Pods green with black streaks, large; seed round . . . . . Type 69

(ii) Seed coat very deep purple, almost black, with silver-white background rarely showing.

† Pods green, black streaks slight . . Type 70
†† Pods green, black streaks heavy . . Type 71

3. Ventral side of standard orange.

(a) Seed coat uniform brownish red, varying from brown to purple, mass colour chocolate red . Type 72

(b) Seed coat with silver-white background having very heavy purple markings . . . Type 73

(c) Seed coat black . . . . . Type 74

C. Red colour diffused over the whole dorsal side of standard and red veins distinct. Type 75

1. Ventral side of standard pale yellow . . .

2. Ventral side of standard orange.

(a) Seed coat with silver-white background, having very heavy purple markings.

(i) Pods green . . . . . Type 76
(ii) Pods green with black streaks . . Type 77

(b) Seed coat with silver-white background almost covered with heavy purple markings . Type 78

(c) Seed coat black . . . . . Type 79

III. Red colour diffused on dorsal side of standard, red veins generally not visible.

A. Red colour existing in patches.

1. Seed coat light fawn with faint brown markings, mass colour light fawn, pods not beaded . . Type 80
2. Seed coat fawn with brown markings, mass colour yellowish brown, pods not beaded . . . Type 81
3. Seed coat brownish fawn, pods beaded . . . Type 82

B. Red colour uniformly covering the dorsal side of standard.

1. Dorsal side of the standard light red . . . Type 83
2. Dorsal side of the standard crimson.
   (i) Ventral side of standard pale yellow.
      a. Pods green with black streaks . . Type 84
      b. Pods black with green streaks . . Type 85
   (ii) Ventral side of standard deep yellow . . Type 86

V. Origin of the types.

Places from where the original seed was obtained.

Type No.	Place	District	Province
1	Khulna	Khulna	Bengal.
2	Jessore	Jessore	,,
3	Tatkon	Yamethin	Burma.
4	Pusa	Darbhanga	Bihar and Orissa.
5	,, ...	,, ...	,,
6	,, ...	,, ...	,,
7	Haveri	Dharwar	Bombay.
..	Wardha	Wardha	Central Provinces.
8	Gorakhpur	Gorakhpur	United Provinces.
	..	..	America.

Type No.	Place	District	Province
10	Nadiad	Kaira	Bombay.
11	Pusa	Darbhanga	Bihar and Orissa.
12	Aligarh	Aligarh	United Provinces.
13	Pusa	Darbhanga	Bihar and Orissa.
14	,,	,,	,,
15	,,	,,	,,
..	Nagpur	Nagpur	Central Provinces.
..	Sepaya	Saran	Bihar and Orissa.
16	Pusa	Darbhanga	,,
17	,,	,,	,,
18	,,	,,	,,
19	Bulandshahr	Bulandshahr	United Provinces.
20	Ahmedabad	Ahmedabad	Bombay.
..	Nadiad	Kaira	,,
21	Aligarh	Aligarh	United Provinces.
22	Pusa	Darbhanga	Bihar and Orissa.
23	,,	,,	,,
24	,,	,,	,,
25	Coimbatore	Coimbatore	Madras.
26	Comilla	Tippera	Bengal.
27	Dacca	Dacca	,,
28	,,	,,	,,
29	Aligarh	Aligarh	United Provinces.
30	Nadiad	Kaira	Bombay.
31	Ahmedabad	Ahmedabad	,,
32	Nagpur	Nagpur	Central Provinces.
..	Murshidabad	Murshidabad	Bengal.
33	Nadiad	Kaira	Bombay.
34	Nagpur	Nagpur	Central Provinces.

Type No.	Place	District	Province
35	Pusa	Darbhanga	Bihar and Orissa.
36	Partabgarh	Partabgarh	United Provinces.
37	Pusa	Darbhanga	Bihar and Orissa.
..	Burdwan	Burdwan	Bengal.
38	Pabna	Pabna	,,
39	Sabour	Bhagalpur	Bihar and Orissa.
40	Mandalay	Mandalay	Burma.
41	Gorakhpur	Gorakhpur	United Provinces.
..	Mandalay	Mandalay	Burma.
42	Pusa	Darbhanga	Bihar and Orissa.
43	Nadiad	Kaira	Bombay.
44	Nagpur	Nagpur	Central Provinces.
45	Pusa	Darbhanga	Bihar and Orissa.
46	,,	,,	,,
47	,,	,,	,,
48	Yeotmal	Yeotmal	Central Provinces.
49	Muttra	Muttra	United Provinces.
50	Pusa	Darbhanga	Bihar and Orissa.
51	Aligarh	Aligarh	United Provinces.
52	Pusa	Darbhanga	Bihar and Orissa.
53	Dharwar	Dharwar	Bombay.
..	Pusa	Darbhanga	Bihar and Orissa.
..	Coimbatore	Coimbatore	Madras.
..	Trichinopoly	Trichinopoly	,,
..	Bangalore	Bangalore	Mysore.
..	Guntur	Guntur	Madras.
54	Sagaing	Sagaing	Burma.
..	Sepaya	Saran	Bihar and Orissa.
..	Mandalay	Mandalay	Burma.

Type No.	Place	District	Province
...	Gorakhpur	Gorakhpur	United Provinces.
55	Pusa	Darbhanga	Bihar and Orissa.
56	Cawnpore	Cawnpore	United Provinces.
...	Coimbatore	Coimbatore	Madras.
57	Pusa	Darbhanga	Bihar and Orissa.
58	,,	,,	,,
..	Samalkot	East Godaveri	Madras.
59	Pusa	Darbhanga	Bihar and Orissa.
60	,,	,,	,,
61	Poona	Poona	Bombay.
62	Nadiad	Kaira	,,
63	Pusa	Darbhanga	Bihar and Orissa.
64	,,	,,	,,
65	,,	,,	,,
66	,,	,,	,,
67	,,	,,	,,
68	Gorakhpur	Gorakhpur	United Provinces.
69	Pusa	Darbhanga	Bihar and Orissa.
...	Bangalore	Bangalore	Mysore.
...	Chindwin	Chindwin	Burma.
70	Wardha	Wardha	Central Provinces.
71	,,	,,	,,
72	Pusa	Darbhanga	Bihar and Orissa.
73	Murshidabad	Murshidabad	Bengal.
74	Gorakhpur	Gorakhpur	United Provinces.
75	Jessore	Jessore	Bengal.
76	Pusa	Darbhanga	Bihar and Orissa.
77	,,	,,	,,
78	,,	,,	,,

Type No.	Place	District	Province
79	Pusa	Darbhanga	Bihar and Orissa.
80	,,	,,	,,
81	Coimbatore	Coimbatore	Madras.
82	Pusa	Darbhanga	Bihar and Orissa.
83	Khulna	Khulna	Bengal.
84	,,	,,	,,
85	,,	,,	,,
86	Guntur	Guntur	Madras.

Some of the types described in this paper have already been mentioned under different numbers or designated by letters in previous annual reports. The correspondence between the type number and the old designations is shown below :—

New number		Previous letter or number
Type 4	=	M
,, 5	=	Type 1 or F
,, 15	=	B or 22-1
,, 16	=	80 or E
,, 24	=	T
,, 25	=	C
,, 41	=	136
,, 50	=	99
,, 51	=	87 or K
,, 59	=	H
,, 64	=	S
,, 66	=	172
,, 69	=	A
,, 72	=	P
,, 73	=	D
,, 79	=	R
,, 80	=	A_2 or WR
,, 82	=	G or 163 or 204

VI. SEED ANALYSIS OF SOME TYPES, 1927-28.

Type No.	Moisture	Ether extract	Crude proteids	Soluble carbohydrates	Woody fibre	Soluble mineral matter	Sand	Total organic nitrogen	Albuminoid nitrogen	Albuminoid ratio
69	8·61	1·15	20·49	59·27	6·35	4·09	0·04	3·28	3·06	3·24
15	8·45	0·79	19·50	58·13	9·36	3·75	0·02	3·12	2·73	3·52
25	8·06	0·83	21·36	56·91	8·98	3·85	0·01	3·42	3·08	3·06
73	8·72	1·29	21·76	57·39	6·96	3·82	0·06	3·48	3·25	2·97
16	8·38	1·08	16·71	61·35	8·73	3·74	0·01	2·67	2·54	4·02
5	7·87	1·16	18·96	60·79	7·42	3·77	0·03	3·03	2·80	3·63
82	7·35	1·04	21·03	59·19	7·35	4·01	0·03	3·36	3·12	3·16
59	8·18	1·08	18·58	59·81	8·48	3·86	0·01	2·97	2·78	3·59
51	7·85	0·87	18·41	61·03	7·90	3·93	0·01	2·95	2·74	3·69
4	7·91	1·17	18·93	61·14	7·08	3·74	0·03	3·03	2·79	3·66
72	8·26	1·25	18·13	61·72	6·87	3·71	0·06	2·90	2·67	3·88
79	7·11	0·78	17·30	61·99	8·97	3·84	0·01	2·77	2·56	3·98

VII. DESCRIPTION OF THE TYPES.

Type 1.—Plants spreading, tall (367 cm.), early. Leaves medium in size (9·01×3·41 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side very pale yellow. Pods scattered, green with or without a few black spots, not beaded, broad, medium in length (6·6 × 1·2 cm.). Seeds round, large, weight of 100 seeds 14·2 grms., seed coat silver-white with fairly heavy brown spots, mass colour silver-white with faint brown tinge.

Type 2.—Plants spreading, medium in height (304 cm.), early. Leaves medium in size (10·10 × 3·42 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side very pale yellow. Pods scattered, green with or without a few black spots, not beaded, medium in size (6·2 × 1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 12·1 grms., seed coat silver-white with fairly heavy brown spots, mass colour silver-white with faint brown tinge.

Type 3.—Plants spreading, medium in height (300 cm.) early. Leaves narrow, medium in length (9·39 × 2·85 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side very pale yellow. Pods scattered, green with or without a few black spots, not beaded, medium in size (6·5 × 1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·5 grms., seed coat brownish fawn.

Type 4.—Plants spreading, tall (345 cm.), late. Leaves medium in size (9·42 × 3·23 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow and "ringed", *i.e.*, with small red dots on margin, ventral side pale yellow having red markings at the base. Pods scattered, green, not beaded, short, narrow (5·5 × 0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·0 grms., seed coat silver-white with numerous small faint grey dots, mass colour silver-white.

Type 5.—Plants erect, medium in height (295 cm.), late. Leaves medium in size (9·95 × 3·44 cm.), midrib purple, faint red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks not "ringed", ventral side pale yellow. Pods crowded, green with black streaks, not beaded, narrow, medium in length (6·3 × 0·7 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·5 grms., seed coat silver-white with numerous small faint grey markings, mass colour silver-white.

Type 6.—Plants spreading with straggling branches, medium in height (264 cm.), early. Leaves medium in size (8·22 × 3·09 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, not "ringed", ventral side pale yellow. Pods scattered, green with black streaks, not beaded, short, narrow (4·4 × 0·7 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·0 grms., seed coat silver-white with numerous small faint grey markings, mass colour silver-white.

Type 7.—Plants spreading, medium in height (259 cm.), early. Leaves short, medium in breadth (7·88 × 3·04 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side pale yellow. Pods scattered, green with black streaks, not beaded, medium in size (6·2 × 0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 10·6 grms., seed coat silver-white with numerous small faint brown dots, mass colour silver-white.

Type 8.—Plants erect, tall (346 cm.), late. Leaves large (10·88 × 4·04 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side pale yellow. Pods scattered, green with or without a few black spots, not beaded, medium in length,

narrow (6·2 × 0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·3 grms., seed coat with light fawn background nearly covered with brown markings, mass colour faint brown.

Type 9.—Plants spreading, medium in height (274 cm.), late. Leaves large (10·95 × 3·89 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side pale yellow. Pods scattered, green with or without a few black spots, not beaded, medium in length, broad (6·8 × 1·4 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 12·8 grms., seed coat brownish fawn.

Type 10.—Plants spreading with straggling branches, tall (335 cm.), early. Leaves broad, medium in length (10·25 × 3·96 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side pale yellow. Pods scattered, green with or without a few black spots, beaded, medium in size (7·0 × 1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·3 grms., seed coat brownish red.

Type 11.—Plants erect, medium in height (313 cm.), late. Leaves medium in size (10·72 × 3·61 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with or without a few black spots, not beaded, short, narrow (5·8 × 0·8 cm.). Seeds lentil-shaped, small weight of 100 seeds 11 grms., seed coat white with grey patch round the hilum hilum brown, mass colour white.

Type 12.—Plants spreading, medium in height (319 cm.), late. Leaves medium in size (8·51 × 3·21 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with or without a few black spots, not beaded, short, medium in breadth (5·5 × 0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·5 grms., seed coat white with grey patch round the hilum, hilum brown, mass colour white.

Type 13.—Plants erect, medium in height (298 cm.), late. Leaves medium in size (10·37 × 3·61 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with or without a few black spots, not beaded (somewhat beaded), medium in size (6·5 × 0·9 cm.). Seeds round, medium in size, weight of 100 seeds 12·2 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 14.—Plants erect, medium in height (300 cm.), late. Leaves medium in size (10·55×3·79 cm.), midrib purple, faint red tinge on young stem and branches

Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods crowded, green with black streaks, not beaded, short, narrow (5·8×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·9 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 15.—Plants erect, medium in height (286 cm.), late. Leaves long and medium in breadth (11·15×3·58 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (5·7×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·3 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 16.—Plants erect, tall (340 cm.), late. Leaves large (10·81×4·0 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow, deep red markings at the base of wings. Pods scattered, green with black streaks, not beaded, broad, medium in length (6·8×1·2 cm.). Seeds round, large, weight of 100 seeds 16·6 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 17.—Plants spreading, medium in height (270 cm.), late. Leaves medium in size (9·45×3·02 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with black streaks beaded, short, narrow (5·4×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·1 grms., seed coat silver-white with numerous small, faint grey marks, mass colour silver-white.

Type 18.—Plants spreading, tall (332 cm.), late. Leaves medium in size (9·78×3·24 cm.), midrib purple, young stem and branches appearing reddish purple. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·4×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 10 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 19.—Plants spreading, medium in height (275 cm.), late. Leaves medium in size (9·34×3·34 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (4·8 ×0·8 cm.). Seeds lentil-shaped, small, weight of 100

seeds 7 grms., seed coat silver-white with numerous small faint grey marks, mass colour silver-white.

Type 20.—Plants spreading, with straggling branches, medium in height (270 cm.), early. Leaves medium in size (8·52×3·5 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with or without a few black spots, beaded, medium in size (6·2×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·3 grms., seed coat silver-white with heavier grey markings than Types 13 to 19 and with brown spots, mass colour silver-white with faint brown tinge.

Type 21.—Plants spreading, medium in height (313 cm.), late. Leaves medium in size (9·68×3·17 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with or without a few black spots, not beaded, short, narrow (5·0×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·0 grms., seed coat with light fawn back-ground nearly covered with brown markings, mass colour faint brown.

Type 22.—Plants erect, medium in height (320 cm.), late. Leaves long and medium in breadth (11·82×3·59 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow with few or no red marks, ventral side deep yellow. Pods scattered, red with green streaks, not beaded, medium in size (7·2×1·0 cm.). Seeds round, medium in size, weight of 100 seeds 13·9 grms., seed coat with fawn background, having brown markings, mass colour brown.

Type 23.—Plants erect, medium in height (300 cm.), late. Leaves broad and medium in length (10·33×4·14 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red markings, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·8×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 12·2 grms., seed coat with fawn background having brown markings, mass colour brown.

Type 24.—Plants spreading, medium in height (288 cm.), late. Leaves medium in size (10·59×3·62 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, narrow (5·6×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 8·8 grms., seed coat uniform greyish fawn.

Type 25.—Plants spreading, medium in height (311 cm.), late, Leaves medium in size (10·17×3·41 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with few or no red marks, ventral

side deep yellow. Pods scattered, green with or without a few black spots, not beaded, medium in length, narrow (6·2×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 8·0 grms., seed coat dark olive-grey nearly covered with black markings.

Type 26.—Plants spreading, tall (374 cm.), early. Leaves medium in size (10·06×3·39 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at base, ventral side pale yellow, red markings present at the base of wings. Pods scattered, green with black streaks, not beaded, broad, medium in length (7·0×1·1 cm.). Seeds round, medium in size, weight of 100 seeds 13·2 grms., seed coat silver-white with heavy grey markings and some brown spots, mass colour silver-white with faint brown tinge.

Type 27.—Plants spreading, tall (327 cm.), early. Leaves medium in size (10·10×3·29 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side pale yellow, red markings present at the base of wings. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·7×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 8·2 grms., seed coat silver-white with heavy grey markings and some brown spots, mass colour silver-white with faint brown tinge.

Type 28.—Plants spreading, medium in height (300 cm.) early. Leaves narrow, medium in length (9·4×2·85 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side pale yellow, red markings present at the base of wings. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·5×1·0 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·4 grms., seed coat silver-white with heavy grey markings and some brown spots, mass colour silver-white with faint brown tinge.

Type 29.—Plants erect, tall (355 cm.), late. Leaves medium in size (9·86× 3·45 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with faint red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, clear red markings present on the wings, on the ventral base of standard and on staminal tube; red dots on the margin of standard. Pods scattered, green with a few small black streaks at the base, not beaded, short, narrow (5·6×

0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·3 grms., seed coat white with grey patch round the hilum, hilum brown, mass colour white.

Type 30.—Plants spreading with straggling branches, medium in height (308 cm.), early. Leaves medium in size (8·86×3·7 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, medium in size (6·5×1·0 cm.). Seeds round, medium in size, weight of 100 seeds 10·4 grms., seed coat silver-white with numerous small faint grey dots, mass colour silver-white.

Type 31.—Plants straggling, medium in height (265 cm.), early. Leaves medium in size (8·21×3·23 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with faint red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·4×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·1 grms., seed coat white background with heavier grey and brown spots than Type 30, mass colour silver-white with faint brown tinge.

Type 32.—Plants spreading, medium in height (250 cm.), early. Leaves small (6·98×3·0 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base. Ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·4 grms., seed coat white with heavier grey and brown spots than Type 30, mass colour silver-white with faint brown tinge.

Type 33.—Plants spreading, medium in height (266 cm.), early. Leaves small (6·89×2·72 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, black, not beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·1 grms., seed coat white with heavier grey and brown spots than Type 30, mass colour silver-white with faint brown tinge.

Type 34.—Plants spreading, medium in height (290 cm.), early. Leaves small (7·48×2·87 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral

side deep yellow. Pods scattered, black, not beaded, medium in size (6·4×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·6 grms., seed coat white with heavier grey and brown spots than Type 30, mass colour silver-white with faint brown tinge.

Type 35.—Plants spreading, tall (343 cm.), late. Leaves broad and medium in length (10·4×3·83 cm.), midrib light purple, young stem and branches appearing reddish purple. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, narrow (5·5×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·4 grms., seed coat uniform light silver-fawn.

Type 36.—Plants spreading, tall (332 cm.), late. Leaves medium in size (9·08×3·37 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base, but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green, with or without a few black spots, not beaded, short, narrow (5·2×0·7 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·1 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 37.—Plants erect, medium in height (305 cm.), late. Leaves long and medium in breadth (11·1×3·51 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base, ventral side deep yellow. Pods scattered, red with green streaks, not beaded, medium in size (6·9×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 7·7 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 38.—Plants spreading, medium in height (298 cm.), early. Leaves medium in size (9·09×3·1 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (5·1×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·4 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 39.—Plants spreading, tall (349 cm.), early. Leaves medium in size (10·11×3·5 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, green with black streaks, beaded, short, narrow (5·6×0·8 cm.). Seeds lentil-shaped, small, weight

of 100 seeds 7·9 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 40.—Plants spreading, medium in height (291 cm.), late. Leaves short and medium in length (7·73×3·03 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (5·8×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 5·7 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 41.—Plants spreading, tall (378 cm.), late. Leaves medium in size (9·15×3·16 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (4·9×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 6·9 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 42.—Plants spreading, tall (343 cm.), late. Leaves medium in size (10·54×3·44 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, black with green streaks, not beaded, short, narrow (5·5×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·5 grms., seed coat light fawn background with brown markings, mass colour fawn to faint brown.

Type 43.—Plants straggling, medium in height (255 cm.), early. Leaves broad and medium in length (8·83×4·11 cm.), midrib light purple, faint red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, green with black streaks, beaded, medium in size (6·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·2 grms., seed coat brownish fawn.

Type 44.—Plants spreading, medium in height (238 cm.), early. Leaves narrow and medium in breadth (8·07×3·0 cm.) midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks,

not beaded, short, medium in breadth (5·6×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 7·6 grms., seed coat brownish fawn.

Type 45.—Plants spreading, medium in height (318 cm.), late. Leaves long, medium in breadth (11·16×3·6 cm.), midrib purple distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, narrow, medium in length (6·0×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 11·6 grms. seed coat brownish fawn.

Type 46.—Plants spreading, medium in height (305 cm.), late. Leaves medium in size (9·96×3·58 cm.), midrib purple, young stem and branches appearing reddish purple. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, narrow (5·1×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·6 grms., seed coat brownish fawn.

Type 47.—Plants erect, medium in height (302 cm.), late. Leaves medium in size (9·55×3·45 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, black, beaded, medium in size (6·5×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 13·7 grms., seed coat brownish fawn.

Type 48.—Plants spreading, medium in height (254 cm.), early. Leaves short and medium in breadh (7·33×3·21 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, black, not beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·5 grms., seed coat brownish fawn.

Type 49.—Plants spreading, tall (335 cm.), late. Leaves medium in size (9·42×3·48 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with or without a few black spots, not beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·4 grms., seed coat with fawn background having brown markings, mass colour light yellow brown.

Type 50.—Plants erect, medium in height (285 cm.), late. Leaves large (11·02×3·83 cm.), midrib light purple, faint red colour on young stem and

branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring at the base, ventral side deep yellow. Pods scattered, red with green streaks, not beaded, short, medium in breadth (5·8×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·8 grms., seed coat with fawn background having brown markings, mass colour light brown.

Type 51.—Plants erect, tall (347 cm.), late. Leaves large (11·44×3·82 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, red with green streaks, not beaded, medium in size (6·2×1·0 cm.). Seeds round, large, weight of 100 seeds 15·3 grms., seed coat with fawn background having brown markings, mass colour yellow brown.

Type 52.—Plants erect, medium in height (300 cm.), late. Leaves medium in size (10·13×3·63 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (5·5×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 8·5 grms., seed coat with fawn background having brown markings, mass colour yellow brown.

Type 53.—Plants spreading, medium in height (235 cm.), early. Leaves medium in size (8·67×3·32 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·0×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·8 grms., seed coat with fawn background having brown markings, mass colour yellow brown or light brown.

Type 54.—Plants spreading, tall (341 cm.), late. Leaves medium in size (8·57×3·62 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (5·0×0·7 cm.). Seeds lentil-shaped, small, weight of 100 seeds 6·4 grms., seed coat with fawn background having brown markings, mass colour yellowish brown.

Type 55.—Plants spreading, tall (356 cm.), late. Leaves medium in size (9·49×3·35 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep

yellow. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·5 grms., seed coat with fawn background having brown markings, mass colour light brown to brown.

Type 56.—Plants spreading, medium in height (304 cm.), late. Leaves narrow, medium in length (9·38×2·92 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, short, narrow (4·7×0·7 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·5 grms., seed coat with fawn background having brown markings, mass colour light brown.

Type 57.—Plants spreading, medium in height (317 cm.), late. Leaves broad, medium in length (10·64×3·88 cm.), midrib purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with faint red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, not beaded, narrow, medium in length (6·0×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·5 grms., seed coat with fawn background having brown markings, mass colour light brown.

Type 58.—Plants spreading, tall (338 cm.), late. Leaves medium in size (9·78×3·64 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers, yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·4×0·9 cm.). Seeds round, small, weight of 100 seeds 8·2 grms., seed coat with fawn background having brown markings, mass colour yellow brown.

Type 59.—Plants erect, tall (326 cm.), late. Leaves medium in size (10·57×3·54 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with faint red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, black with green streaks, not beaded, short, narrow (5·8×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·3 grms., seed coat with fawn background having brown markings, mass colour light brown.

Type 60.—Plants erect, medium in height (315 cm.), late. Leaves large (11·36×3·85 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, black with green streaks, not beaded, medium in size

(6·0×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·7 grms., seed coat with fawn background having brown markings, mass colour yellow brown.

Type 61.—Plants spreading, medium in height (223 cm.), early. Leaves small (8·0×2·81 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, black with green streaks, not beaded, short, narrow (5·5×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 6·9 grms., seed coat with fawn background having brown markings, mass colour reddish brown.

Type 62.—Plants spreading with straggling branches, medium in height (300 cm.), early. Leaves broad and medium in length (8·73×4·15 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with clear red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, medium in size (7·2×1·0 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·8 grms., seed coat brownish red, varying from light to dark red.

Type 63.—Plants spreading, tall (338 cm.), late. Leaves broad and medium in length (9·94×4·03 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·4×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 11·0 grms., seed coat dark olive-grey.

Type 64.—Plants spreading, medium in height (322 cm.), late. Leaves medium in size (9·59×3·45 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with faint red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow. Pods scattered, black with green streaks, beaded, short, medium in breadth (5·9×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·5 grms., seed coat dark olive-grey.

Type 65.—Plants erect, medium in height (285 cm.), late. Leaves medium in size (10·1×3·2 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins radiating from base but having no ring of diffused red colour at the base, ventral side deep yellow, red markings present at the base of wings. Pods scattered, green with black colour diffused over whole surface leaving green streaks, beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of

100 seeds 9·7 grms., seed coat dark olive-grey, nearly covered with black markings.

Type 66.—Plants spreading, tall (341 cm.), late. Leaves medium in size (9·39×3·45 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with distinct red veins and a ring of diffused red colour at the base, ventral side pale yellow having red markings at the base. Pods scattered, green with or without a few black spots, not beaded, short medium in breadth (5·0×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·2 grms., seed coat with light fawn background nearly covered with brown markings, mass colour faint brown.

Type 67.—Plants spreading, tall (355 cm.), late. Leaves medium in size (10·58×3·76 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with distinct red veins and a ring of diffused red colour at the base, ventral side pale yellow, having red markings at the base. Pods scattered, green with or without a few black spots, beaded, short, narrow (5·9×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·8 grms., seed coat with light fawn background nearly covered with brown markings, mass colour faint brown.

Type 68.—Plants erect, medium in height (315 cm.), late. Leaves large (11·60×3·84 cm.), midrib green, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with distinct red veins and a ring of diffused red colour at the base, ventral side deep yellow having red markings at the base of wings. Pods scattered, green with or without a few black spots, not beaded, medium in size (6·4×0·9 cm.). Seeds lentil-shaped, large, weight of 100 seeds 11·5 grms., seed coat with fawn back ground having brown markings, mass colour light brown.

Type 69.—Plants erect, medium in height (323 cm.), late. Leaves large (12·04×4·54 cm.), midrib light purple, faint red colour on young stem and branches. Flowers yellow, dorsal side of standard yellow with distinct red veins and a ring of diffused red colour at the base, ventral side deep yellow having red markings at the base. Pods scattered, green with black streaks not beaded, large (8·5×1.1 cm.). Seeds round, large, weight of 100 seeds 17·8 grms., seed coat with fawn background having brown markings, mass colour light brown.

Type 70.—Plants spreading, short (203 cm.), early. Leaves short and medium in breadth (7·24×3·21 cm.), midrib green, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow with red veins and a ring of diffused red colour at the base, ventral side deep yellow, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with a few small black streaks, short, narrow (5·2×0·7 cm.). Seeds lentil-

shaped, medium in size, weight of 100 seeds 9·5 grms., seed coat silver-white background almost covered with heavy purple markings.

Type 71.—Plants spreading, short (180 cm.), early. Leaves medium in size (8·22×3·31 cm.), midrib light purple, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow with red veins and a ring of diffused red colour at the base, ventral side deep yellow, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered green with heavy black streaks, short, narrow (5·0×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 8·2 grms., seed coat with silver-white background almost covered with heavy purple markings.

Type 72.—Plants spreading, tall (335 cm.), late. Leaves medium in size (10·36×3·37 cm.), midrib green, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard yellow with red veins and a ring of diffused red colour at the base, ventral side pale orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with red streaks, beaded, short, medium in breadth (5·5×1·0 cm.). Seeds round, medium in size, weight of 100 seeds 10·9 grms., seed coat uniform brownish red, varying from brown to deep purple, mass colour chocolate-red.

Type 73.—Plants spreading, tall (382 cm.), late. Leaves medium in size (10·41×3·74 cm.), midrib purple, young stem and branches appearing reddish purple. Flowers yellow, dorsal side of standard yellow with red veins and a ring of diffused red colour at the base, ventral side orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with black streaks, not beaded, short, narrow (4·8×0·8 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 7·5 grms., seed coat silver-white background having very heavy purple markings.

Type 74.—Plants erect, medium in height (315 cm.), late. Leaves medium in size (10·34×3·57 cm.), midrib light purple, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow with red veins and a ring of diffused red colour at the base, ventral side pale orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered green with black streaks, not beaded, short, narrow (5·3×0·8 cm.). Seeds lentil shaped, medium in size, weight of 100 seeds 9·5 grms., seed coat black.

Type 75.—Plants spreading, medium in height (250 cm.), early. Leaves narrow and medium in length (9·17×2·67 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard having distinct red veins and red colour diffused over the whole dorsal surface and not restricted to a half ring at the base, ventral side pale yellow, red colour on the wings marked, red markings on the ventral base of standard also present. Pods scattered, green with black streaks, not beaded, medium in size (6·2×0·9 cm.).

Seeds lentil-shaped, medium in size, weight of 100 seeds 11·6 grms., seed coat silver-white with fairly heavy and large brown spots, mass colour silver-white with faint brown tinge.

Type 76.—Plants spreading, tall (346 cm.), late. Leaves broad and medium in length (10·27×4·01 cm.), midrib green, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow having distinct red veins and diffused red colour, ventral side orange, red colour on the wings marked, red markings on the ventral base of standard also present. Pods scattered, green with or without a few black spots, beaded, short, medium in breadth (5·8×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·8 grms., seed coat with silver-white back-ground having very heavy purple markings.

Type 77.—Plants spreading, tall (359 cm.), late. Leaves medium in size (9·83×3·79 cm.), midrib purple, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow having distinct red lines and diffused red colour, ventral side orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 9·3 grms., sead coat with silver-white background having very heavy purple markings.

Type 78.—Plants spreading, tall (358 cm.), late. Leaves medium in size (10·19×3·69 cm.), midrib purple, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow having distinct red veins and diffused red colour, ventral side orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·5×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 10·6 grms., seed coat with silver-white back-ground, almost covered with heavy purple markings.

Type 79.—Plants spreading, tall (350 cm.), late. Leaves medium in size (10·10×3·67 cm.), midrib purple, young stem and branches appearing bluish purple. Flowers yellow, dorsal side of standard yellow having distinct red veins and diffused red colour, ventral side orange, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with black streaks, beaded, short, medium in breadth (5·4×0·9 cm.). Seeds lentil-shaped, medium in size, weight of 100 seeds 12·6 grms., seed coat uniform black.

*Type 80.**—Plants spreading, tall (340 cm.), late. Leaves medium in size (9·0×3·4 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, red colour diffused in patches on the dorsal side of the standard,

* Type 80 is the A_2 wilt resistant type described in the Scientific Monograph No. 7 of the Imperial Council of Agricultural Research [McRae and Shaw, 1933] on wilt resistance.

but red veins generally absent, red markings at the base of wings present. Pods scattered, green with black streaks, not beaded, short, narrow, (5·7×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·7 grms., seed coat light fawn with faint brown markings, mass colour light fawn.

Type 81.—Plants spreading, short (220 cm.), early. Leaves short, medium in breadth (7·92×3·19 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, red colour diffused in patches on dorsal side of standard, but red veins generally absent, red markings at the base of wings present. Pods scattered, green with black streaks, not beaded, short, medium in breadth (5·0×0·9 cm.). Seeds lentil-shaped, small, weight of 100 seeds 7·3 grms., seed coat with fawn background having brown markings, mass colour yellowish brown.

Type 82.—Plants spreading, tall (359 cm.), late. Leaves medium in size (9·77×3·34 cm.), midrib purple, young stem and branches appearing reddish purple. Flowers yellow, red colour diffused in patches on the dorsal side of the standard but red veins generally absent, red marking at the base of wings present. Pods scattered, green with black streaks, beaded, short, narrow (4·8×0·8 cm.). Seeds lentil-shaped, small, weight of 100 seeds 9·2 grms., seed coat brownish fawn.

Type 83.—Plants spreading, tall (338 cm.), early. Leaves medium in size (9·49×3·09 cm.), midrib light purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard uniformly light red, clear red markings present at the base of wings and on the ventral base of standard. Pods scattered, green with red streaks, not beaded, broad, medium in length (6·6×1·3 cm.). Seeds round, medium in size, weight of 100 seeds 14·6 grms., seed coat silver-white with fairly heavy brown spots, mass colour silver-white with faint brown tinge.

Type 84.—Plants spreading, medium in height (283 cm.), early. Leaves medium in size (9·16×3·58 cm.), midrib purple, faint red tinge on young stem and branches. Flowers yellow, dorsal side of standard uniformly deep crimson, ventral side pale yellow, red colour on the wings marked, red markings on the ventral base of standard also present. Pods scattered, green with black streaks, not beaded, broad, medium in length (7·0×1·2 cm.). Seeds round, large, weight of 100 seeds 16·1 grms., seed coat silver-white with fairly heavy brown spots, mass colour silver-white with faint brown tinge.

Type 85.—Plants spreading, tall (338 cm.), early. Leaves narrow, medium in length (9·47×2·95 cm.), midrib light purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard uniformly deep crimson, ventral side pale yellow, red colour on wings marked, red markings on the ventral base of standard also present. Pods scattered, green with black colour diffused over whole surface leaving green streaks, not beaded, broad, medium in length

(7·2×1·2 cm.). Seeds round, medium in size, weight of 100 seeds 12·7 grms., seed coat silver-white with grey markings and some brown spots.

Type 86.—Plants spreading, medium in height (270 cm.), early. Leaves medium in size (9·23×3·53 cm.), midrib purple, distinct red colour on young stem and branches. Flowers yellow, dorsal side of standard uniformly deep crimson, ventral side deep yellow, red colour on wings marked. Pods scattered, green with black streaks, not beaded, narrow, medium in length (6·6×0·7 cm.). Seeds lentil-shaped, small, weight of 100 seeds 8·1 grms., seed coat fawn background having reddish brown markings, mass colour brown.

VIII. REFERENCES.

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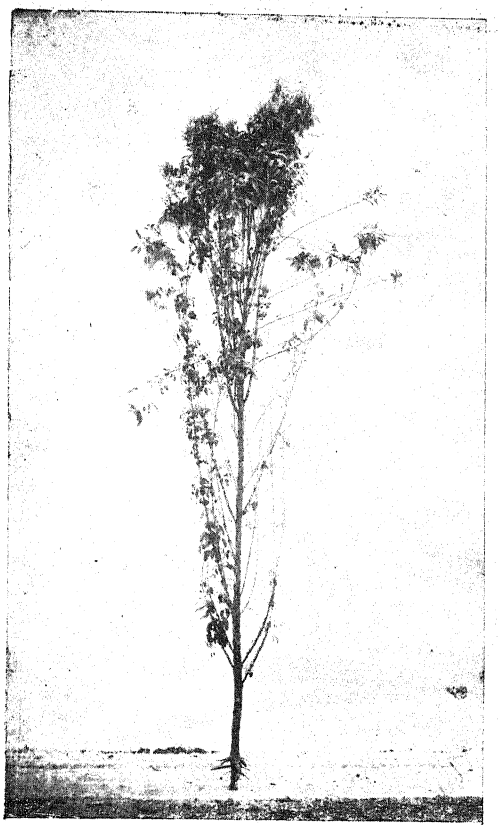


Fig. 1—T. 5.

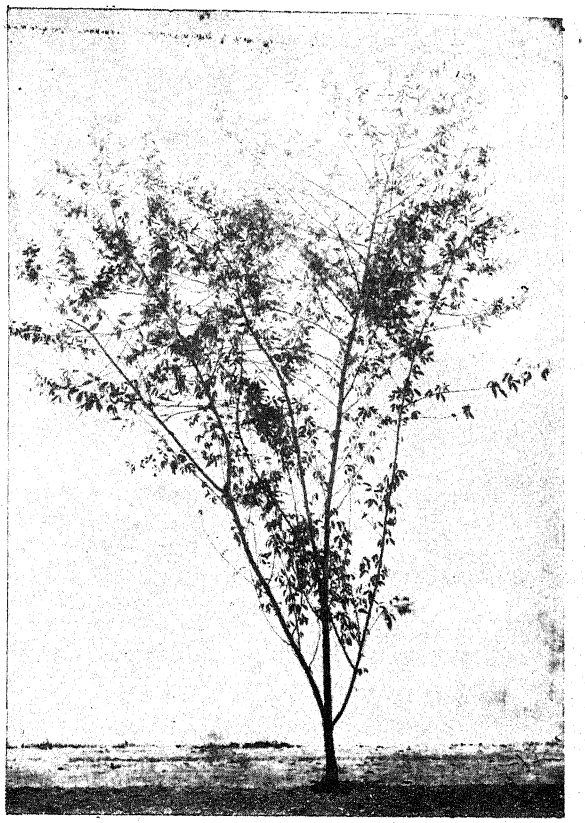


Fig. 2—T. 80.

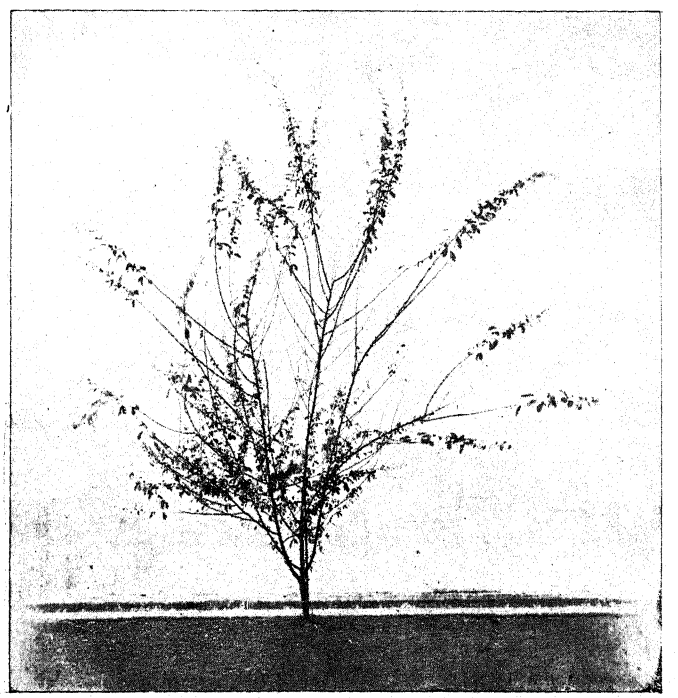
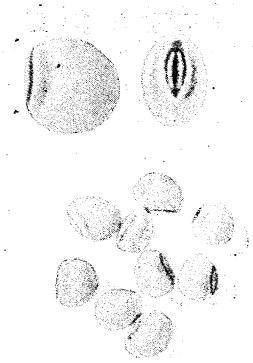


Fig. 3—T. 62.

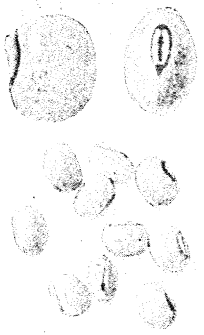
T. 1.
T. 13.
T. 27.
T. 36.
T. 71.
T. 72.
T. 26.
T. 53.
T. 66.
T. 68.
T. 75.
T. 76.
T. 82.
T. 83.
T. 84.
T. 88.

T. 67. T. 68. T. 72. T. 83. T. 22.

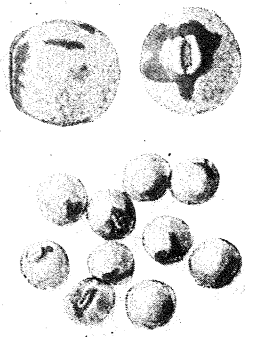
T. 17. T. 69. T. 85. T. 47. T. 48.



T. 11.



T. 4.



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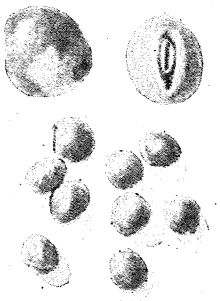

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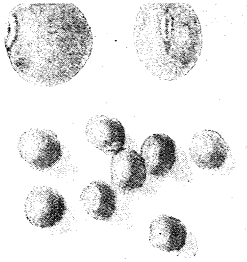

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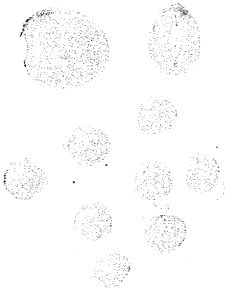

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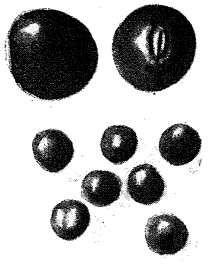

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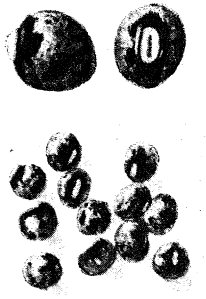

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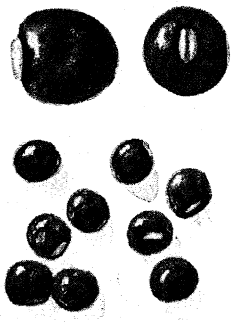

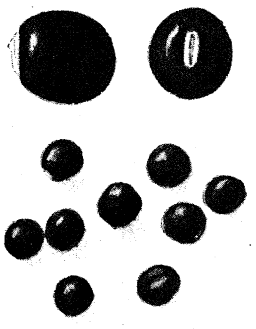

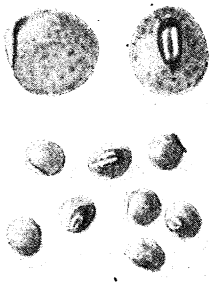

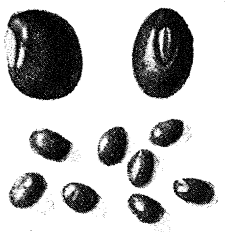

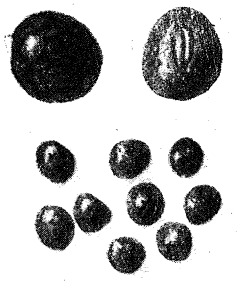

OBSERVATIONS ON THE CYTOLOGY OF THE SUGARCANE.

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(With Plates V-VIII)

I. Introduction.

The cytology of Saccharum has received attention from Franck [1911], Kuwada [1915], and more recently from Bremer [1923]. In a comprehensive scheme of the study of anatomy, ontogeny and the cytology of the sugarcane, Artschwager, Brandes and Starrett [1929], have studied the varieties U. S. 875 and U. S. 1694, and have described in detail the development of the flower and seed in these varieties.

Bremer's [1923] outstanding work has thrown valuable light on the chromosome behaviour in the various forms of Saccharum and certain inter-varietal and inter-specific hybrids, as also on the taxonomic relationship of *Saccharum arundinaceum* and *S. munja*. During the arrowing season 1930-31 and 1931-32 a study of fertilization and embryogeny in the sugarcane was taken up and the results, as far as available, are reported in this paper. An attempt was also made to determine the chromosome number in the following varieties of *S. officinarum* grown in India, *viz.*, Vellai, Shamshara, Poovan, Chittan and Puri. Since meiosis was expected to be regular in it, *S. spontaneum* (Coimbatore) was included to serve as a control, and later on Co. 205 was added because of its interest as a seedling of a cross between *S. officinarum* and *S. spontaneum*.

II. Material and methods.

All the material was collected from the canes grown at the Thick Cane Area of the Imperial Sugarcane Station, Coimbatore, except Poovan which was collected from Kuniamuthur, a village about six miles away.

For fixing the flowers, arrows slightly before the 'flag' stage were selected. As the spikelets at the top of the arrows generally contained bright yellow anthers in which fully formed pollen grains were found, flowers from the lower portions of the arrows were taken. These contained pale yellow or whitish anthers which on staining with Belling's [1921] Acetocarmine were found to contain a large number of dividing pollen-mother cells. The best time for fixing the material was found to be between 9 and 11 A.M.

The following fixatives were tried :—(1) La Cour's [1929]. One per cent. chromic acid.—90 c.c. ; potassium bichromate—1 grm.; sodium sulphate—0·5 grm. ; urea—1 grm. ; 5 per cent. acetic acid—10 c.c. ; 2 per cent. osmic acid—15 c.c. ; distilled water—45 c.c. (2) Carnoy's [Lee, 1928] :—Chloroform—3 parts ; absolute alcohol—6 parts ; acetic acid—1 part, and (3) Allen's modification of Bouin's fluid [Lee, 1928] :—Picric acid (saturated solution)—75 c.c. ; formaldehyde—25 c.c.. ; acetic acid—5 c.c. ; chromic acid—1·5 grms. ; urea—2 grms. The first and second fixatives were not found to be as satisfactory as the third, which was therefore employed for fixing most of the material for study.

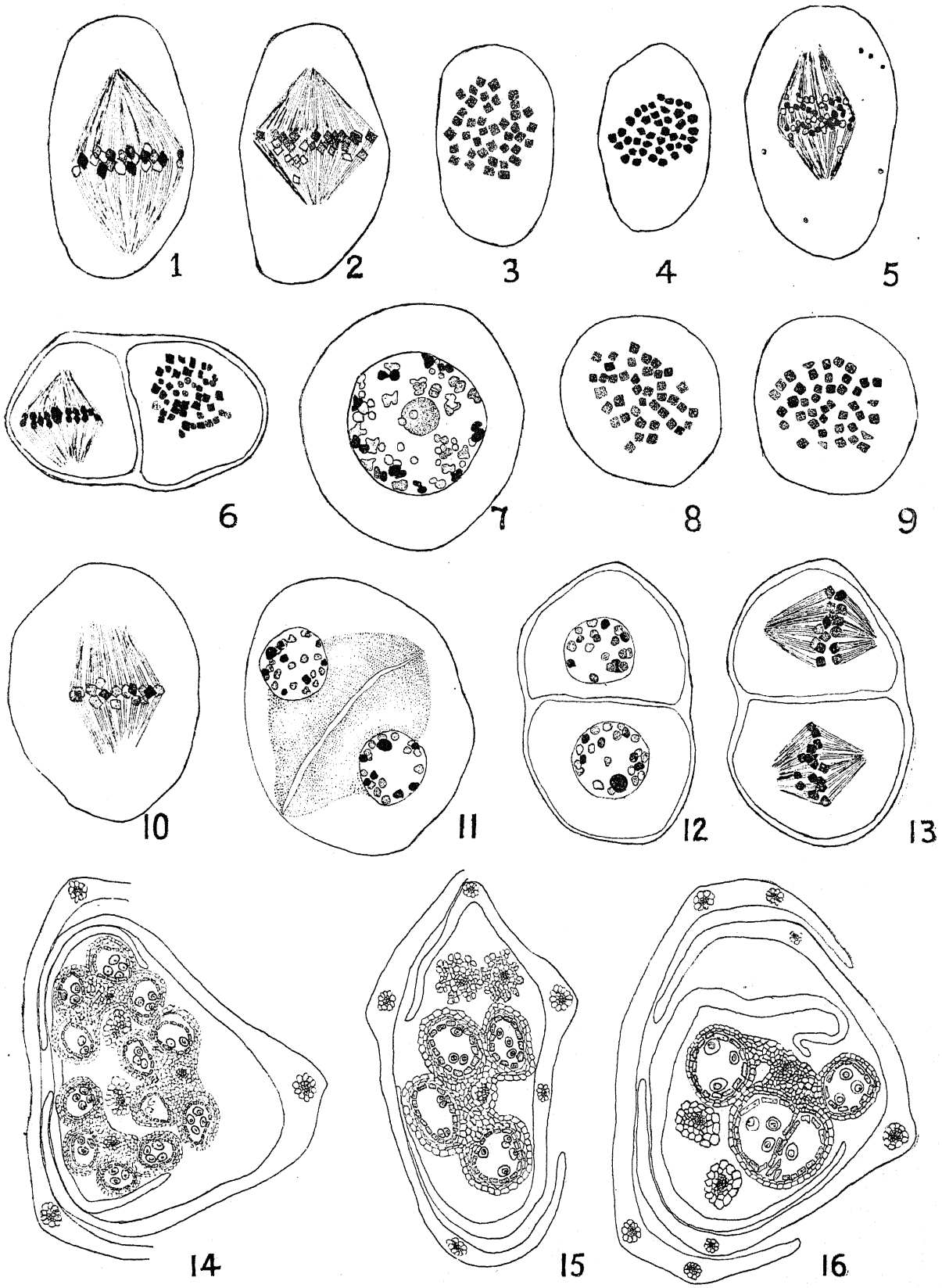
The flowers were kept in the fixative for 24 hours and after washing in running water for the same period, were run up through the usual alcohol grades, cleared in xylol, and finally embedded in paraffin (54°C.). The callus hairs were removed from flowers as they were being run up through the alcohol grades and wherever ovaries had to be sectioned, the glumes also were removed to facilitate cutting. Sections of anthers were cut 8 to 12 μ thick and of ovaries 15 to 20 μ thick and stained with Heidenhain's Iron Alum Haematoxylin. To ensure the removal of all traces of picric acid before staining, slides were left for about half an hour in a saturated solution of lithium carbonate in 70 per cent. alcohol.

In order to corroborate the chromosome numbers obtained from microsporocytes, root tips were also fixed in some of the varieties. Sections were cut 8 to 10 μ thick and stained in Heidenhain's Iron Alum Haematoxylin.

III. Chromosome numbers.

(*a*) *Vellai*.—The morphological characters of Vellai were compared with the published botanical descriptions of Otaheite [Earle, 1928 ; Deerr, 1921 ; Rosenfeld, 1927], and it was found that they agreed in most respects. It was thought that a cytological study of Vellai might reveal features which will help in its identification.

Chromosome counts were made from polar views of the metaphase equatorial plates. One of these is represented in Plate V, figure 3, and forty chromosomes are seen, probably all bivalents. In Plate V, figure 4, are seen forty-one chromosomes, evidently due to irregularity in the heterotypic division. In not a few counts, the numbera 41 and 42 were encountered, but as in the majority of counts, the number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was 40, it may be taken that the haploid number in Vellai is 40. This number could not be confirmed in counts of the bivalents at the diakinesis stage, as in the preparations examined suitable stages could not be found. In Plate V, figures 2 and 5, certain chromosomes are seen travelling in advance, while Plate V, figure 6 shows irregular homeotypic division in one of the diads.

An examination of the somatic chromosomes as also of the bivalents in the diakinesis stage of the microsporocytes will need to be made before Vellai and Lahaina [Otaheite] could be compared satisfactorily.

(*b*) *Shamshara.*—This cane has been described by Woodhouse, Basu and Taylor [1915], and they consider it to be very similar to Benaresia Nepali. Earle [1928] in the annotated list of cane varieties mentions Shamshara as equal to Otaheite. Like the other Paunda canes, this cane is also probably an introduced tropical cane, but according to Deerr [1921] under the name Samsara has travelled out again from India as an Indian cane.

Chromosome counts were taken from diakinesis and also from equatorial plates. In Plate V, figure 7, are seen 33 pairs and 14 univalents at diakinesis. In Plate V, figure 8, 40 chromosomes are represented while Plate V, figure 9 shows 38 bivalents and 4 univalents. The heterotypic division of the microsporocytes would thus appear to be irregular while in Plate V, figure 13 is shown the side-views of the metaphases of the homeotypic divisions where, in one of the diads, the arrangement of the chromosomes is somewhat irregular.

The suppression of one and sometimes two anthers was noticed to be a common occurrence in this variety. Plate V, figures 15 and 16 show these abnormalities, while a normal case is represented in Plate V, figure 14. Yet another abnormality which was sometimes met with is shown in Plate VI, figure 17 where two pairs of styles are seen.

From the above, it will be noted that Shamshara differs from Vellai in some respects. A few morphological differences were also noticed between the two. As diakinesis and a few other stages were not available in Vellai, it has not been possible to compare these two varieties in all cytological details.

(*c*) *Poovan.*—In morphological characters Poovan resembles Keli and Hottai Kabbu. These varieties are probably identical, but grown under different local names. Poovan also resembles Pundia in certain characters. As none of the above-mentioned varieties, with the exception of Poovan, flowered, their identification could not be confirmed by a cytological study of the flowers.

Chromosome counts were made from diakinesis and also from polar views of the equatorial plates. Poovan shows very irregular meiosis, in fact the most irregular of all the varieties examined. Lagging chromosomes were invariably met with in the metaphase and the anaphase while univalents were common in diaki-

nesis and polar views of metaphase. In Plate VI, figure 20, 23 bivalents and 39 univalents are seen, while Plate VI, figure 19 represents 33 bivalents and 13 univalents. Plate VI, figure 18 shows 34 gemini and 12 unpaired chromosomes in diakinesis.

The somatic chromosomes are represented in Plate VI, figure 23 and were found to be 78. As it was very difficult to make exact counts in root tips, reliance has to be placed mainly on the counts in meiosis. From the various counts the haploid chromosome number in Poovan would appear to be about 40.

(*d*) *Chittan.*—Barber [1916] in his classical work on Indian sugarcanes mentions that from the detailed morphological study of Chittan, Karun and Kaludai Boothan, it seemed probable that they are closely related to one another, there being some ground for supposing that Chittan, a striped cane, is the oldest and that Karun (Claret) and Kaludai Boothan (green with blush of pink) have at some time arisen as sports from it. With a view to study the cytology of these forms, spikelets in the desired stages of microsporogenesis, and root tips were fixed in Chittan and Kaludai Boothan. It was proposed to take up Karun later.

Counts could not be made from the polar views of metaphase plates, as the chromosomes were noticed to be very closely packed together. This closely packed nature of the chromosomes extended to practically all the stages, including homeotypic division. The prophases and diakinesis stages, however, were clear enough and the gemini could be distinctly traced in the latter. Plate VI, figure 24 shows 40 gemini in diakinesis while irregular anaphase is represented in Plate VI, figure 26. The somatic chromosomes are shown in Plate VI, figure 27. The haploid chromosome number in Chittan may be tentatively taken as about 40.

In the preparations of Kaludai Boothan also, the chromosomes were found to be closely packed together and no good diakinesis stages could be seen to enable the counting of chromosomes.

(*e*) *Puri.*—This cane has been described by Woodhouse, Basu and Taylor [1915]. Deerr [1928] grew Creole and Caliph's Cane alongside Puri, and after a very careful examination of their morphological characters came to the conclusion that they were identical in all respects. The main observations on the cytology of Puri are detailed below.

Chromosome counts were made from the diakinesis and also from the polar views of metaphase. Meiosis in Puri was found to be more regular than Vellai, Shamshara, Poovan and Chittan. In Plate VI, figure 31, forty bivalents are seen in the polar view of the metaphase of the microsporocyte, while 40 gemini are also seen in the diakinesis stage (Plate VI, figure 29). In Plate VI, figures 30 and 33 are shown the regular metaphase and telophase respectively, while Plate VI, figure 32 represents a more or less regular anaphase. In the preparations examined, no laggards were observed and similarly no univalents were seen either in the side or

polar views of the metaphase. The homeotypic division was also noticed to be regular (Plate VI, figure 35). In some sections darkly stained bodies probably chromatin granules were observed in the cytoplasm. These might be extruded chromatin.

The following has appeared in an abstract of Bremer's [1932] sixth paper on the cytology of sugarcane. "The cane known as Yellow Egyptian, thought to be the same as Creole is discussed in a separate section; 81 was established as the somatic number with 39 bivalents and 3 univalents, at metaphase of the reduction division. This leads the author to regard this cane as a hybrid between the Mungo Indian cane with 41 and a noble cane with 40. The characteristic remains of the nucleolus referred to above was also observed here, a phenomenon which in *S. officinarum* is never observed. This affords further indication of the Indian and hybrid origin of the Creole cane."

In the preparations of Puri the present writers never came across the characteristic remains of the nucleolus which, according to Bremer [1923], is typical of the Indian sugarcanes. The only indications of chromatin in the cytoplasm were very small, darkly stained granules—smaller in size than gemini. These are of course in no way comparable in size to the long membranous body in the cytoplasm usually near one of the poles noticed and figured by Bremer [1923] in the Indian sugarcanes.

Creole could not be examined cytologically by the present writers, as it did not flower at Coimbatore.

(*f*) *Saccharum spontaneum* (*Coimbatore*).—Barber [1915] mentions *S. spontaneum* as a variable species in India. Hole [1911] also found that this species varied greatly according to its habitat, there being three oecological forms, *viz.*, (1) the most xerophilous form found on dry sandy soil, (2) the most hygrophilous form found in swamps and marshy places, and (3) a form intermediate between the first and second, usually found in loam. This difference in habitat was associated with a difference in growth. As there were numerous intermediate forms, Hole [1911] did not think it advisable to define these as different sub-species or varieties. Owing to the slight difference of the African forms—sub-species *aegyptiacum*, variety *aegyptiacum* from the Indian plants examined by him, Hole [1911] thought that a more complete knowledge of the African plant will prove *aegyptiacum* to be merely one of the several oecological forms.

There seems to be little doubt, however, that at least certain of the forms of *S. spontaneum* differ from one another fundamentally. For instance, Bremer [1923 and 1925] who studied two of these forms cytologically, found that the Java *S. spontaneum* (Glagah) had 56 haploid chromosomes, while Glagah Tabongo—the *S. spontaneum* of North Celebes—had 40 haploid chromosomes. The collection at Coimbatore includes about nine forms and the variation in the quality of the juice

is from about 2 per cent. sucrose to as much as over 8 per cent. [Venkatraman, 1930].

S. spontaneum (Coimbatore) affords yet another example of a form with different chromosome number. The haploid chromosome number was found to be 32. Chromosome counts were made from the polar views of the equatorial plates and also from diakinesis. Meiosis was seen to be very regular. In Plate VII, figures 38, 39 and 40, are shown the polar views of the metaphase in which 32 chromosomes could be counted beyond any doubt. Similarly 32 pairs could also be counted at diakinesis (Plate VII, figure 36). In Plate VII, figures 37 and 41, are represented the side-views of metaphase and anaphase. The homeotypic division was also noticed to be regular, as seen in Plate VII, figure 44. Thirty-two chromosomes are seen in equatorial plates of each of the diads. The somatic number, as seen in Plate VII, figure 46, was found to be 64. The haploid chromosome number of *S. spontaneum* (Coimbatore) may therefore be taken as 32.

(*g*) *Co. 205.*—This seedling belongs to the F_1 progeny of the cross Vellai *S. spontaneum* [Venkatraman and Rao, 1928], and the writers were informed that the *S. spontaneum* employed was the Coimbatore form. Bremer [1923], while working on the cross between *S. officinarum* and the Java form of *S. spontaneum* found that the resulting seedlings had an increased chromosome number. For example, Kassoer which is supposed to be a cross between Black Cheribon and Glagah (Java) when examined cytologically was found to show 68 chromosomes and since Black Cheribon has 40 haploid chromosomes and Glagah 56, one would expect Kassoer to have 48 haploid chromosomes. But instead, it possesses 68. Obviously the diploid chromosome number 136 could be arrived at by a fusion of twice the haploid chromosome numbers of the female parent, *i.e.*, 2 (40) and the haploid number of the male parent, *i.e.*, 56 ; the alternative possibilities being that (1) the reduction division had failed to take place and (2) that during fertilization the *S. officinarum* chromosomes undergo longitudinal fission. Bremer [1923] thinks the latter explanation as the more probable one, because the division stages of the egg mother cell in *S. officinarum* observed by him, always pointed towards reduction division. A similar phenomenon of the increased chromosome number in seedlings was also found by him in the cross between *S. officinarum* and the Indian cane Chunnee. The following seedlings of this cross, *viz.*, P.O.J. 181, P.O.J. 213 and P.O.J. 920 were found to have chromosome numbers varying between 62 and 64, giving the diploid number of approximately 124 to 128 which is obtained by adding the diploid number in *S. officinarum, i.e.*, 2 (40) and the haploid number of Chunnee, *i.e.*, 46 to 48.

It seemed worthwhile to ascertain if the seedlings of *S. officinarum* and the Coimbatore form of *S. spontaneum* also had an increased chromosome number on

the same basis as Kassoer and P.O.J. 181, P.O.J. 213 and P.O.J. 920. Chromosome counts of Co. 205 were made from polar views of the metaphase and also from diakinesis. The metaphase plates in Plate VII, figures 48 and 49, show the 56 number very clearly and similarly Plate VII, figure 47, shows 56 pairs at diakinesis. In Plate VII, figure 50, is represented the side-view of the metaphase. While most of the side-views of metaphase and anaphase were found to be regular, in Plate VII, figure 51, is shown an irregular anaphase. In Plate VII, figure 52 is represented the regular homeotypic divisions, where 56 chromosomes were counted in each diad.

From the very clear polar views of the equatorial plates observed in many sections, the haploid number in Co. 205 may be taken as 56. Root tips were not fixed in Co. 205, but as the haploid chromosome number was conclusively found to be 56, the somatic number would therefore be 112. This number can be arrived at by adding the diploid number of Vellai to the haploid number of the pollinating parent—*S. spontaneum* (Coimbatore), *i.e.*, 2(40) *plus* 32 which equals 112. This would confirm Bremer's [1923] observations on the increase in chromosome numbers in the seedlings of the cross between *S. officinarum* and (1) Glagah and (2) Chunnee.

IV. Chromosome numbers in relation to size of nuclei.

Definite relationship has been established between chromosome numbers and systematic position of the various species in certain genera of plants. According to Bremer [1925], there is in *Saccharum* a close relationship between the size of the nuclei of microsporocytes and the haploid chromosome number. The method adopted by him was to measure the largest and the smallest diameter of the nuclei of a large number of microspore mother cells in the diakinesis stage and then to calculate the radius from the mean diameter. When the radius was raised to the third power, it was found that r^3 was proportional to the haploid chromosome number, *e.g.*, for Glagah, which has 56 haploid chromosomes, r^3 was 588 and 539 or not far from 560, *i.e.*, ten times the haploid number. Similarly for four varieties of *S. officinarum* with 40 as their haploid chromosome number, r^3 was 403, 405, 426 and 384. The same ratio held good for *S. officinarum* × *S. spontaneum* crosses as also for Glagah Tabongo (N. Celebes), Tanangge and Hitam Rokan. The deviation from the above ratio was large in the case of Chunnee, Ruckree II and Katha, amounting as it did to 18 per cent., and was greater still in *S. arundinaceum* and *S. munja*, *i.e.*, 30 per cent. and was about the same as in *Erianthus*. The haploid chromosome number in *S. arundinaceum* and *S. munja* was also the same as in *E. ravennae* and *E. japonicus*. This fact and the same size of their nuclei led Bremer [1925] to conclude that *S. arundinaceum* and *S. munja* should belong to the genus *Erianthus*.

In view of the conclusions referred to above, it was decided to calculate r^3 in the varieties studied in this paper and to ascertain its relation to the haploid chromosome number. Only such nuclei were measured as were unmistakably in the diakinesis stage. The data are presented in Table I.

TABLE I.

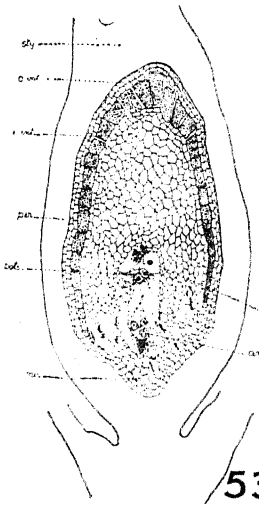
Size of nuclei of microsporocytes at diakinesis and haploid chromosome numbers.

Name of variety	No. of nuclei measured	Mean diameter in microns	Mean radius in microns	r^3	Haploid chromosome number
Poovan	40	9·84	4·92	119	About 40
Chittan	51	13·2	6·6	287	About 40
Puri	21	11·76	5·88	203	40
S. spontaneum (Coimbatore)	26	9·04	4·5	91	32
Co. 205	20	10·4	5·2	141	56

For the above measurements, the varieties Vellai, Kaludai Boothan and Shamshara were not included, as their microspore mother cells were not in the typical diakinesis stage. It will be seen from the above that r^3 deviates 70 per cent., 27 per cent., 50 per cent., 72 per cent. and 75 per cent. from ten times the haploid chromosome number in the varieties, Poovan, Chittan, Puri, *S. spontaneum* (Coimbatore) and Co. 205 respectively. It is difficult to explain so large a deviation unless it be that the pollen mother cells and their nuclei in the above preparations had become smaller owing to imperfect fixation, but this would appear to be hardly likely as no plasmolysis was noticed. Before any definite opinion can be expressed, it will be necessary to collect more data on these as well as on other varieties.

V. AN ABNORMAL EMBRYO-SAC.

In a normal sugarcane embryo-sac (1) the egg and the synergids are towards the micropylar end, (2) the polar nuclei immediately above the egg, and (3) the antipodal complex towards the chalazal end. In the course of the study of fertilization and embryogeny in sugarcane, an abnormal embryo-sac was met with in the cross Vellai × C. A. C. 87 and is shown in Plate VIII, figures 53 and 54. The antipodal nuclei are seen to be situated near the micropylar end while the polars and the egg are towards the opposite end. Since this is unusual, the writers



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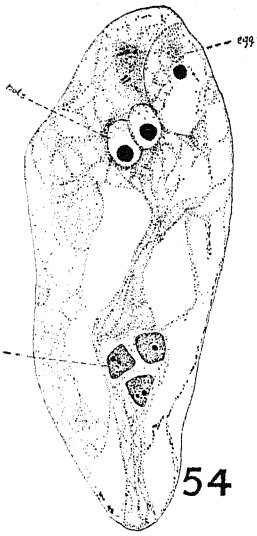

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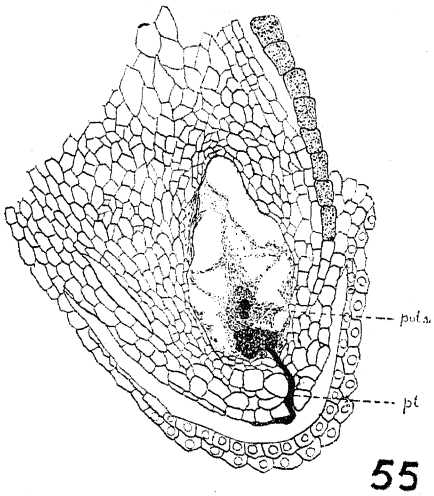

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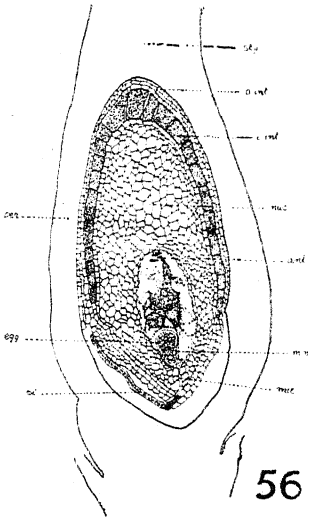

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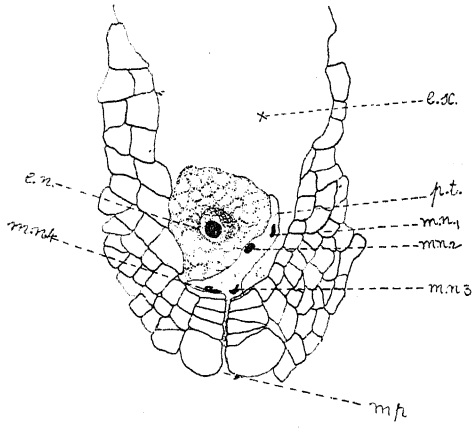

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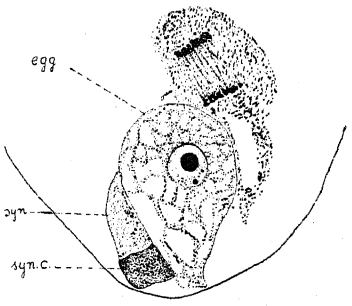

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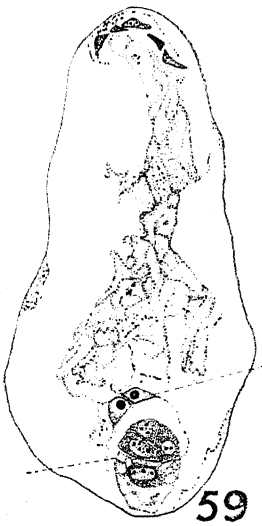
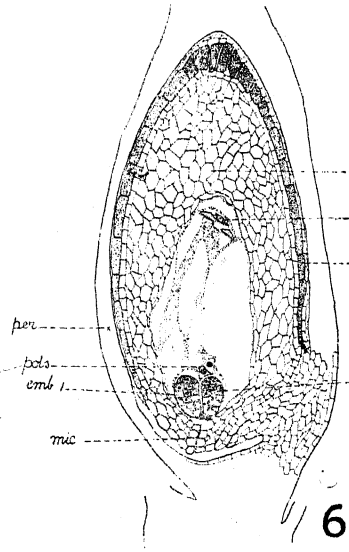
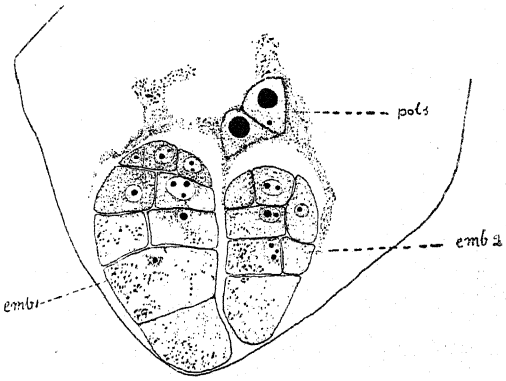


59



60



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(For explanation see page 56.)

repeatedly and carefully examined the sections in question to make sure that no mistake was made in the identification of the structures. From their shape and size as compared with the normal cases, it appeared certain that the nuclei towards the micropylar end were the antipodals. The polar nuclei were also identified with certainty. Above the polars was seen the nucleus of the egg, and though the outline of the cell was not very clear, from its position with reference to other components of the embryo-sac, it would appear that the structure in question was the egg. Some dense cytoplasm with chromatin granules was seen near the egg which probably represents the remains of the disorganized synergids.

VI. Fertilization.

In a previous publication on the growth of sugarcane pollen tube *in vitro* [Dutt, 1929], it was stated that under favourable conditions, the sugarcane pollen tube might reach the ovary in about three to four hours. This has now proved to be an under-estimate by about three hours from the detailed studies that were taken up later.

Flowers of the variety Vellai were fixed at definite intervals after pollinating with C. A. C. 87 pollen in order to ascertain (1) the time taken by the pollen tube to reach the embryo-sac, (2) subsequent stages of fertilization and (3) embryogeny. The interval was half an hour from five to ten hours after pollination and half a day from one to nine days.

The pollen tube was found to have reached the embryo-sac $7\frac{1}{2}$ hours after pollination in the cross Vellai × P.O.J. 1410 (Plate VIII, figure 56), and this was also confirmed in the cross P.O.J. 2725 × Co. 285 (Plate VIII, figure 55), where the pollen tube was seen entering the micropyle seven hours after pollination. In the preparations of the cross Vellai × C. A. C. 87, however, the exact time taken by the pollen tube to reach the embryo-sac could not be determined, but in two sections the primary endosperm nucleus was found to be in division eight hours after pollination. One of these is shown in Plate VIII, figure 58, in which the primary endosperm nucleus is seen to be in telephase. This would indicate that the pollen tube would have entered the embryo-sac some time before eight hours after pollination.

In the preparations, fusing sex nuclei were not observed, but in sections of the ovaries collected one day after pollination, the endosperm was usually found in the coenocytic stage, while fertilized egg was seen to be dividing not later than two days after pollination.

VII. Generative nuclei.

In a previous paper [Dutt and Krishnaswami, 1932], it was mentioned that two male nuclei were noticed to have been formed inside the sugarcane pollen

grains twenty-four hours before shedding. This condition has since been observed in nineteen more sugarcane varieties as also in *S. munja*. Very young flowers of C. A. C. 87 were stained in aceto-carmine and on examination, two male nuclei were seen to have been formed about five days before shedding. The observations on the occurrence of four male nuclei in certain sugarcane varieties were continued and the cross Vellai × B. 3412 was effected with the specific object of ascertaining the significance of the occurrence of more than two male nuclei in the sugarcane.

Further observations were made on the migration of the two male nuclei from the pollen grain to the pollen tube. Percentages of tubes into which the nuclei had migrated were worked out in the artificially cultured pollen of Co. 285 and *S. spontaneum*. Counts were made at intervals of thirty minutes, one hour, two hours and three hours after the first germination. In the one hour old cultures, the nuclei had migrated in 40 per cent. of the tubes, while in three-hour ones, the migration of the nuclei had taken place in 80 per cent. of the tubes. In certain thirty minutes and one-hour pollen tubes, one generative nucleus was noticed to be inside the grain while the second had migrated some distance into the tube. Such tubes were counted in one culture (Co. 285, one hour old culture), and were found to be six per cent. of the total germinations. No clear relationship was found between the migration of the nuclei and the length of the pollen tube. In three hours old cultures, certain tubes (about 400μ long) were found with the nuclei still inside the pollen grain. These tubes had probably stopped growing at an early stage. On the other hand, both the nuclei had migrated in certain ten minutes old cultures, the tubes being only 108μ long. Further, in a tube which was 1800μ long (P. 671, six hours old culture), the nuclei had travelled only 450μ from the grain, while it was not uncommon to find the nuclei at the extreme tip in much shorter pollen tubes.

As for the occurrence of more than two nuclei in other plants, De Mol [1923] who worked on *Hyacinthus orientalis* was successful in obtaining plurinuclear pollen grains experimentally. In most cases four of these nuclei were present, but five to eight have been observed by him. He further observed that these plurinuclear pollen grains (1) could be more easily induced to germinate in cane sugar solutions, (2) frequently sprouted while still inside the unopened anther and (3) formed wide, somewhat bladder-shaped pollen tubes. When four male nuclei were present in the tubes, one was sometimes lying at some distance from the other three and was larger than these. Such a large nucleus was frequently lying in the end part of the tube, while the other somewhat smaller ones had remained in the pollen grain or had just entered into the pollen tube. The observations

made on the four male nuclei in the sugarcane differ from those of De Mol [1923] in the following respects. (1) More than four male nuclei have not been observed so far, (2) the pollen grains and tubes with four male nuclei are not abnormal in any way and behave exactly as the normal ones in their germination capacity as well as in the nature of their pollen tubes, (3) the four male nuclei in the tube were observed to occur in pairs, either both the pairs moving closely together or at some distance from each other, and (4) the size of all the male nuclei was more or less the same. The usual size of the male nucleus inside the pollen tube is about 8 to 10μ long, though small globular nuclei about 5μ in diameter and very long vermiform nuclei measuring 26μ in length were occasionally observed.

Out of the several sections of ovaries of Vellai that had received B. 3412 pollen, only in one four male nuclei were observed inside the embryo-sac. This section is shown in Plate VIII, figure 57. It will be seen that the pollen tube has just reached the embryo-sac and the tip has not yet burst to liberate the four male nuclei, all of which are still seen to be inside the pollen tube. In this case, if the nuclei had been set free, they would clearly be all from the same pollen tube and there will be no uncertainty as was felt in *Gagea lutea* where Nemec [1912] was not sure whether the two sperm nuclei fusing with the egg and the other two fusing with the nucleus of the embroy-sac were derived from the same pollen tube or from two separate ones. Similarly Ishikawa [1918] who came across an aberrant case in *Oenothera mutans*×*Oe. pycnocarpa* found two male nuclei near the egg nucleus while the third was just coming in contact with the large pole nucleus. As the pollen tube was lacking in the preparation, he was unable to decide whether the extra nuclei were brought about by intrusion of two sets of sperm nuclei due to an attack of two pollen tubes on a single embryo-sac or by the production of excess generative nuclei in the male gametophyte.

As to the significance of the occurrence of four male nuclei the following points deserve mention. According to Coulter and Chamberlain [1909] this may have no further significance than that any active cell may be induced to divide by favourable conditions. Certain other investigators, however, are of opinion that two male nuclei fertilize the egg nucleus leading to the origin of a plant possessing triploid nuclei. Nemec [1912] often observed triple fusion in the egg nucleus in *Gagea lutea*.

De Mol [1923] observed two types of abnormal pollen grains in *Hyacinthus orientalis*, *viz.*, (1) Plurinuclear pollen grains, and (2) Large, globular, diploid pollen grains. He is inclined to the view that triploid seedlings owe their origin to the fertilization of a haploid egg nucleus by two haploid male nuclei or one diploid nucleus.

VIII. Ovogenic apogamy.

While examining the preparations of the cross Vellai × C. A. C. 87 for stages in the development of the embryo, it was observed in two sections that a few divisions had taken place in the egg, while the characteristic coenocytic stage of the endosperm was absent. One of these is shown in Plate VIII, figure 59. It will be seen that the polar nuclei are intact and have not fused. This particular ovary was fixed two days after pollination. While giving the details about fertilization, it has been mentioned that in the sugarcane, (1) the pollen tube reaches the embryo-sac in about seven hours, (2) the endosperm is in the free nuclear stage in about twenty-four hours after pollination, and (3) the fertilised egg usually starts division not later than two days. If, therefore, these embryo-sacs had received the male nuclei, there would normally have been a formation of the endosperm. From the above, it would appear that the embryos in question have in all probability arisen parthenogenetically.

IX. Polyembryony.

Many cases of polyembryonyo have been noticed in Angiosperms, the well-known examples being those of *Citrus aurantium* and other plants. The extra embryos arise either from the cells of the embryo-sac other than the egg—apogamy —or by sporophytic budding. In the latter, the cells of the nucellus and those of the integument are usually involved, while in the former, cases are known where every cell within the embryo-sac produced an embryo. Though the synergids and antipodal cells have frequently been noticed to give rise to embryos, cases where endosperm cells have formed embryos are very rare. Such cases according to Sharp [1926] are open to grave doubt. Woodworth [1930] working on *Alnus rugosa* has, however, reported a case of an embryo which apparently originated from endosperm material.

The occurrence of polyembryony in Gramineæ has been reported in the following cases :—Kuwada [1910] noticed in rice "an abnormal formation of two embryo-sac mother cells" which would suggest the possibility of polyembryony in rice. Komura [1922] reported the formation of two plumules in a rice seed after germination, and Rodrigo [1926] found that one seed of the rice variety Inintiw produced two plumules and two primary radicles. In the 214,000 rice seeds germinated by him he noticed only one which produced two plumules. Jones [1928] reports a case of two hybrid plants apparently "identical twins" derived from one fertilized egg in a cross of the rice varieties Yosemite and Nimai Kawa Mochi. Hansen [1920] found that double-germ grains occurred in Mahndorfer winter wheat at the rate of 1 ; 10000, in Mahndorfer oats at the rate of 1 : 20000 ; and in 7 per cent. of the seeds

in one strain of rye. Nishimura [1923] noticed polyembryony in *Poa pratensis*. Ayyangar and Krishnaswami [1930] have reported the occurrence of polyembryony in *Eleusine coracana*.

While examining the sections of the cross Vellai×C. A. C. 87, two embryos in one embryo-sac were noticed. The section is shown in Plate VIII, figures 60 and 61. The embryos are lying side by side, one of them is normal while the other has apparently arisen as a result of the fertilization of one of the synergids by the second male nucleus. The two polar nuclei, it will be seen, have not yet fused. The second embryo in this case, *i.e.*, the one that has developed from the fertilized synergid, may also be regarded as normal and not apogamous. Over 100,000 seeds are germinated every year at the Thick Cane Area in connection with sugarcane breeding, and the writers have not so far come across a seed which produced either two plumules and two radicles, two plumules with a single radicle or a single plumule with two radicles.

X. Discussion.

Stages in meiosis pointed to the haploid number in Vellai, Shamshara, Poovan, Chittan and Puri to be about forty. All these canes apparently belong to the species *S. officinarum* and their chromosome number would appear to bring them into a line with the " noble " varieties studied by Bremer [1924].

In the cytoplasm darkly stained granules—about the size of univalents—were noticed in the varieties Vellai and Puri. These granules were the only indication of extruded chromatin. The long membranous body in the cytoplasm, usually near one of the poles, which according to Bremer [1923] is characteristic of the canes belonging to *S. Barberi*, was not noticed in any of the canes studied in this paper including *S. spontaneum*. The preparations of Co. 205 were rather dark, due to La Cour's [1929] fixative, but as far as could be seen, no chromatin in the cytoplasm, in the shape of the characteristic remains of the nucleolus, was noticed. It may be stated that the extruded chromatin noticed in Vellai and Puri was found to be an exception and not the rule. Extruded chromatin has been observed by various workers in other plants. Church [1929] noticed it in *Panicum festuca*, *Avena sativa* and *Spartina*, while considerable cytoplasmic chromatin was seen by Beck and Horton [1932] in all the three species of Bromus studied by them, particularly in *B. marginatus* in which a continuous mass of chromatin was found adhering to the cell wall. Often there seemed to be as much material in the mass as on the spindle. The continuous mass in *B. marginatus* would remind one, in certain respects, of the long membranous body noticed by Bremer [1923] in the Indian canes. Extruded chromatin has also been frequently observed in Rosa by Erlanson [1929].

According to Bremer [1925], the chromosome numbers found in Saccharum clearly point to 10 (or possibly 5) being the original number of the Maydeae and Andropogoneae. The haploid number 32 in the Coimbatore form of *S. spontaneum* would mean that this form is a dysploid, a term employed by Jeffrey [1925] to denote "non-multiploid variation in number of the nuclear chromosomes". Several instances of dysploidy in Gramineae have been given by Church [1929]. For instance, a series of 9, 8, 7, and 10 in the genus *Panicum*, and counts of 9 and 11 in *Paspalum*, though the well-established number in the latter genus is 10. If the haploid number in the Indian forms be taken as 48, then the haploid numbers of at least certain of the Saccharum forms, *viz.*, *S. spontaneum* (Coimbatore), *S. officinarum*, *S. spontaneum* (Glagah Tabongo), *S. Barberi* and *S. spontaneum* (Java) with 32, 40, 40, 48, and 56 chromosomes respectively, would form a series with 8 as the basal number. This is only a suggestion based on the occurrence of 32 haploid chromosomes in the Coimbatore form of *S. spontaneum* and may perhaps need to be revised as more data become available on other forms of *Saccharum*. The evidence as at present available on the majority of *Saccharums* points strongly to 10 (possibly 5) being the basal number, but as mentioned above in the case of *Panicum*, it is not unusual to find multiples of different basal numbers in the same genus. Of the euploid series in Gramineae, some of the most interesting are those in which, according to Longley [1932], the chromosome numbers in the perennial forms are twice as many as in the annual forms, *viz.*, *Sorghum halepensis* and *Sorghum sudanensis*.

The occurrence of four male nuclei has been found to be not uncommon in at least certain of the sugarcane varieties. Dispermatic fertilization is probably not the cause of increased chromosome number in the species hybrids in *Saccharum* as the chromosome counts indicate that a doubling has taken place only in the chromosomes of the female parent and not of the male parent. In one of the seedlings of the cross P.O.J. 2875 and Glagah, at Pasoeroean it was found that the chromosome number was 220, which would mean that the chromosomes in both the parents had doubled. This might perhaps be a case in which the chromosomes of the female had split longitudinally during fertilization and had also received two male nuclei.

In certain plant genera an increase in chromosome number has been found to be associated with an increase not only in the size of the cells but also in the size of plants. Sharp [1926] in discussing the relationship between tetraploidy and gigantism states that "the tetraploid mutants are frequently characterised by an unusually large size, not only in the plant as a whole, but also in its anatomical constituents. Winkler [1916] on *Solanum*; Tupper and Bartlett [1916] on *Oenothera stenomeres*. Not all cases of gigantism are associated with tetraploidy. In a form of *Primula sinensis* [Gregory, 1909 and 1914], it is associated with an increase in

the size of the chromosomes and not the number. Sinoto [1925] reports that in *Plantago japonica*, a giant form, the number of chromosomes is one-half than that in the ordinary *P. major*, var. *asiatica*". Denham [1924] found that the cotton plants having 26 chromosomes were on the whole much larger than those with 13 chromosomes. Erlanson [1929] measured the diameter of the microsporocytes of Rosa at diakinesis and found that the diploid types had the smallest cells and the hexaploid the largest, although there was considerable overlapping between the cell sizes at diakinesis in tetraploids and hexaploids. Heilborn [1927] compared the size of pollen tetrads in Draba and found that on the whole the size of the cells increases with the rise in chromosome number. Bremer [1925] found that the cube of the radius of the microsporocyte nucleus at the diakinesis stage was about ten times the haploid chromosome number. In similar measurements made in the varieties studied in this paper, this ratio was not found to hold good. It may, however, be stated that the nuclei of *S. spontaneum* were decidedly smaller in size than those of *S. officinarum*. Further, the three varieties of *S. officinarum*, *viz.*, Poovan, Chittan and Puri, all having the same haploid chromosome number differ from one another in the size of their microsporocyte nuclei. Tahara [1915] found that in the genus *Chrysanthemum*, different species with nine chromosomes had nuclei of very different dimensions.

A cytological examination of the various species of *Saccharum* and allied genera would help in deciding the systematic position of these forms. From morphological view point, Hooker [1897] mentions in the "Flora of British India", 'I find no characters whereby to distinguish *Erianthus* from *Saccharum* except that given above (glume IV awned), which is all but illusory, and a re-examination of both genera may lead to a better disposition of their species under two or more genera or sections'. In fact Bremer [1925] after a very careful examination of cytological data came to the conclusion that *S. munja* and *S. arundinaceum* should be classified under the genus Erianthus.

The abnormal embryo-sac having the antipodals towards the micropyle and the egg towards the opposite end, was the only one of its kind met with in an examination of the sections of over 600 ovaries comprising seven different crosses. The egg of an embryo-sac situated in the abovementioned unusual manner, would by normal fertilization give rise to embryos whose hypocotyle would point towards the chalaza, instead of the micropyle. The writers never came across any such embryo in the large number of sections examined. Such embryos, however, have been reported by Woodworth [1930] in *Alnus rugosa*, which according to him had originated apogamously from the antipodal cells.

Most of the varieties of *S. officinarum* are probably of hybrid origin. Lagging univalents were often met with and sometimes extruded chromatin was also noticed.

As is well known "the presence of unpaired or univalent chromosomes is one of the most striking indications of a hybrid". The occurrence of polyembryony and apogamy would lend support to the above supposition, as according to Ernst [1918] hybridization is the initial cause of meiotic irregularities, apomixis, polyembryony, etc.

XI. Summary.

The haploid chromosome number in Puri, Vellai and Shamshara was found to be 40, and about 40 in Poovan and Chittan.

The Coimbatore form of *S. spontaneum* was found to have 32 haploid chromosomes, while Co. 205, a seedling of the cross between Vellai and *S. spontaneum* (Coimbatore), had 56.

The size of the nuclei of the microsporocytes was not found to be definitely proportional to the haploid chromosome number.

An abnormal embryo-sac was met with in the cross Vellai×C. A. C. 87 in which the antipodal cells were situated towards the micropyle and the egg and polars towards the chalaza.

The pollen tube was found to have reached the embryo-sac seven hours after pollination. The endosperm was seen to be in the coenocytic stage one day after pollination and the division of the egg had commenced two days after pollination.

A pollen tube containing four male nuclei was seen inside the embryo-sac in the cross Vellai×B. 3412.

Two cases of probable parthenogenetic origin of embryos were noticed.

A case of polyembryony was observed in the cross Vellai × C. A. C. 87. The second embryo had probably arisen from a fertilized synergid.

The occurrence of dysploidy in the genus Saccharum and the increase in the chromosome number in the species hybrids is discussed.

We are indebted to Mr. M. K. Krishnaswami for help in taking morphological notes on certain sugarcane varieties and also in the culturing of sugarcane pollen.

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XIII. Explanation of plates.

Except as otherwise stated, all figures were drawn with a Leitz periplanatic eyepiece No. 20 and 1/12 achromatic oil-immersion objective, with the aid of Zeiss Abbe's Camera Lucida at stage level. They were reduced two-thirds in reproduction.

PLATE V.—*Vellai.*

Fig. 1.—Regular heterotypic metaphase.
Fig. 2.—An irregular heterotypic metaphase.
Fig. 3.—Heterotypic metaphase, polar view, showing forty bivalents.
Fig. 4.—Heterotypic metaphase, polar view, showing 41 chromosomes, probably 39 bivalents and two univalents.
Fig. 5.—Heterotypic anaphase; note lagging chromosomes and extrusions.
Fig. 6.—Homeotypic divisions; polar view of the equatorial plate in one of the diads and side-view in the other.

Shamshara.

Fig. 7.—Diakinesis, 33 pairs and 14 univalents. Reconstructed from two sections.
Fig. 8.—Heterotypic metaphase, polar view, 40 bivalents.
Fig. 9.—Same as above, but with 38 bivalents and 4 univalents.
Fig. 10.—Regular heterotypic metaphase.
Fig. 11.—Interkinesis, diad split nearly completed.
Fig. 12.—Diads.
Fig. 13.—Homeotypic divisions, metaphase somewhat irregular in one of the diads.
Fig. 14.—Cross section of a flower, showing 3 anthers and 2 styles.
Fig. 15.—Same as above, showing only one anther, the other two being suppressed.
Fig. 16.—Same as above, but partition wall in one of the locules incomplete.

PLATE VI.

Fig. 17.—Cross section of the flower of Shamshara showing two pairs of styles.

Poovan.

Fig. 18.—Diakinesis, showing 34 bivalents and 12 univalents.
Fig. 19.—Heterotypic metaphase, polar view; 33 bivalents and 13 univalents.
Fig. 20.—Same as above, but with 23 bivalents and 39 univalents.
Fig. 21.—Heterotypic metaphase somewhat irregular.
Fig. 22.—Heterotypic anaphase, with lagging univalents.
Fig. 23.—Somatic plate, showing 78 chromosomes.

Chittan.

Fig. 24.—Diakinesis, showing 40 pairs.
Fig. 25.—Heterotypic metaphase, polar view. The chromosomes ars closely packed together.
Fig. 26.—Heterotypic anaphase with lagging univalents.
Fig. 27.—Somatic plate, showing 78 chromosomes.

Puri.

Fig. 28.—Prophase. Note the protuberance on the nucleolus.
Fig. 29.—Diakinesis, showing 40 pairs.

Fig. 30.—Heterotypic metaphase ; side view.
Fig. 31.—Heterotypic metaphase ; polar view, showing 40 bivalents.
Fig. 32.—Heterotypic anaphase.
Fig. 33.—Heterotypic telophase. Note the extruded chromatin.
Fig. 34.—Interkinesis ; diad split almost completed.
Fig. 35.—Homeotypic divisions ; 40 bivalents in each of the diads.

PLATE VII.—*Saccharum spontaneum* (Coimbatore).

Fig. 36.—Diakinesis ; showing 32 pairs.
Fig. 37.—Heterotypic metaphase.
Fig. 38.—Heterotypic metaphase, polar view, showing 32 bivalents.
Fig. 39.—Same as above in another microsporocyte.
Fig. 40.—Same as above in a third microsporocyte.
Fig. 41.—Heterotypic anaphase.
Fig. 42.—Interkinesis ; diad split not yet commenced.
Fig. 43.—Diads.
Fig. 44.—Homeotypic divisions ; 32 chromosomes in each diad.
Fig. 45.—Pollen grains just released from the tetrad stage.
Fig. 46.—Somatic plate, showing 64 chromosomes.

Co. 205.

Fig. 47.—Diakinesis ; showing 56 pairs. Reconstructed from two sections.
Fig. 48.—Heterotypic metaphase, polar view showing 56 bivalents.
Fig. 49.—Same as above in another microsporocyte.
Fig. 50.—Heterotypic metaphase.
Fig. 51.—Heterotypic anaphase, showing lagging univalents.
Fig. 52.—Homeotypic divisions, 56 chromosomes in each diad. Lower diad reconstructed from two sections.

PLATE VIII.

(In this plate the figures were reduced to one-half in reproduction.)

Fig. 53.—Vellai × C. A. C. 87. Longitudinal section of the ovary showing an abnormal embryo-sac. Note the occurrence of the antipodals towards the micropylar end and of the egg and polars towards the opposite end. Reconstructed from two sections. *sty*, style ; *o int*, outer integument ; *i int*, inner integument ; *per*, pericarp ; *pols*, polars ; *mic*, micropyle ; *ant*, antipodals. (Leitz eye-piece No. 6, objective No. 4.)

Fig. 54.—Embryo-sac in above, enlarged. (Leitz eye-piece No. 10, objective No. 7.)

Fig. 55.—P.O.J. 2725 × Co. 285. Pollen tube entering the micropyle. Seven hours after pollination. (Zeiss eye-piece No. 10, objective No. 40.)

Fig. 56.—Vellai × P.O.J. 1410. Pollen tube inside the embryo-sac. 7½ hours after pollination. *m n*, male nucleus ; *pt*, pollen tube. (Leitz eye-piece No. 6, objective No. 4.)

Fig. 57.—Vellai × B. 3412. Showing the pollen tube inside the embryo-sac with four male nuclei. (Zeiss eye-piece No. 5, objective No. 100.)

Fig. 58.—Vellai × C.A.C. 87. Showing the first division of the primary endosperm nucleus; telophase. Eight hours after pollination, *syn*, synergid; *syn c*, synergidal cap. (Leitz eye-piece No. 6, objective No. 1/12.)

Fig. 59.—Vellai × C.A.C. 87. Showing ovogenic apogamy. *emb*, embryo. (Leitz eye-piece No. 15, objective No. 4.)

Fig. 60.—Vellai × C.A.C. 87. Showing Polyembryony. (Leitz eye-piece No. 6, objective No. 4.)

Fig. 61.—Same as above, embryos enlarged. (Leitz eye-piece No. 6, objective No. 1/12.)

A BIOCHEMICAL STUDY OF THE FORMATION OF THE OIL IN NIGER SEED (*GUIZOTIA ABYSSINICA*).

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(With one text-figure)

I.—Introduction.

The fatty acids which occur in nature as glycerides in the fats and oils contain carbon atoms ranging from C_4 to C_{24} inclusive. The more prevalent fatty acids in the vegetable kingdom generally contain an even number of carbon atoms. Acids with an uneven number of carbon atoms or cyclic acids are comparatively rare. Acids with C_{16} and C_{18} like palmitic, stearic, oleic, linolic, etc., form the major portion of a vegetable fat.

Another striking feature in these fatty acids is the position of the double bond in the molecule of unsaturated acids. In the $C_nH_{2n-2}O_2$, *e.g.*, myristoleic, oleic or erucic it is in the 9 : 10 position from the CH_3 group ; so that in oleic acid it is just in the centre of the molecule. In the linolic or linolenic acids one of the double bonds is just in the 9 : 10 position from either end of the molecule.

Again each different species has a distinctly different kind of fat. Yet the fat of each species is fairly constant in composition though the species may be grown in widely differing localities.

These facts suggest that there is some common mechanism in the formation of these fats in nature. Whatever mechanism is propounded to explain the synthesis of fats it must explain (as Armstrong and Allen [1924] put it) :—

(1). The formation in certain cases of acids consisting of all the lower members of the series.

(2). The formation of C_{18} acids, sometimes together with varying proportions of C_{16} and C_{20} acids.

(3). The formation of oleic acid and the position of the double bond in the middle of the chain.

(1). Fats are synthesised in plants and in animals from carbohydrates, the unit attacked being the primary or basic C_{18} complex of starch which workers in the field of carbohydrate chemistry now consider to be the nucleus of most polysaccharides, rather than the simple C_6 unit of glucose. The C_{18} unit consisting of three C_6 units united through oxygen atoms, undergoes internal re-arrangement, oxidation and reduction, at which moment the rupture of the oxygen bridge and the joining of carbon to carbon might take place.

The product would probably be stearic acid, oleic acid appearing at a later stage. The formation from this of homologous acids higher and lower, both saturated and unsaturated, with an even number of carbon atoms might be explained perhaps by the process of oxidation and aldol condensation.

(2). Second type of fat synthesis is that in which the mechanism is such as to lead to the formation of all the lower members of the series with an even number of carbon atoms, the action taking place in a solution of simple carbohydrate (sugar not starch). The simplest explanation of such a series appears to be the building up of the acids from one another by repeated aldol condensation. In this case the possibility of a progressive far reaching degradation from C_{18} acid to the lower acids must not be excluded as an alternative.

Armstrong and Allen [1924] also refer to the theory put forward by Nencki for the butyric fermentation of lactic acid, thus :—

$CH_3.CH(OH).COOH \longrightarrow CH_3.CHO + HCOOH$

Lactic acid — Acetaldehyde + Formic acid.

$CH_3.CHO + H.CH_2.CHO \longrightarrow CH_3.CHOH.CH_2.CHO$

Two mols. aldehyde

aldol condensation

$CH_3.CHOH.CH_2.CHO \longrightarrow CH_3.CHOH.CH_2.COOH$

Aldehyde — oxidation — Butyric acid.

Higher fatty acids should be formed by a further union of aldehyde molecule and ensuring reduction so that this butyric fermentation would represent the first stage of a reaction which gives rise to the higher fatty acids with an even number of carbon atoms.

3. The third theory (referred to by Armstrong and Allen [1924]), is that glucose is resolved into a C_3 unit and these C units are condensed to fatty compounds or

substances approximating to them with 6 and 9 carbon atoms—such units immediately combine with themselves to form C_{12} or C_{18} molecule. In such a synthesis oleic acid might be the penultimate stage; we have difficulty, according to this theory, in accounting for the ethenoid bond in the centre of the chain in any other fashion. It would appear that whatever may be the mechanism of synthesis there is a tendency to form compounds with C_{18} atoms produced, it may be, from three molecules of glucose, either as such or in the form of starch. The other fatty acids may be formed by secondary action from the C_{18} molecule; *viz.*, by the well-known β oxidation. There is comparatively little work on this line of study. Gerber is perhaps the pioneer in this line of research. He studied the olive seed and came to the conclusion that the formation of fat is accompanied by the disappearance of mannitol and proteins. Le Clerè du Sablon [1893] has shown that in almonds carbohydrates are first brought in the seed and subsequently transformed into oil. Another important observer is Eyre [1924]. He has shown that in the flax seed the accumulation of oil is as rapid as three per cent. of the (dry) seed per day. The unsaturation of the oil increases only at the end of the development of the seed.

Finally as regards the nature of the agents which are responsible for the changes that the seed material undergoes only a few facts are known. The resting seed especially rich in oil contains an enzyme (lipase) which is supposed to bring about the hydrolysis of the fat so as to liberate free fatty acids which are then in their turn broken down into still smaller units of carbohydrates which are necessary for the newly-developing plant. Similarly it has been shown that an enzyme is present in a developing seed which has the property of bringing about synthesis of fats from the free acid and glycerine.

With a view to solve at least some of the problems described above a detailed study of the composition of the niger seed at various stages of formation and the nature of the oil that is accumulating was taken up. The results obtained were of the following nature.

It was found that soluble carbohydrates are the precursors of all the other substances which are finally stored in the seed. Hexoses as well as small quantities of pentoses are brought into the developing seed which are then transformed into fatty acids, higher carbohydrates and proteid matter. So that finally in the resting stage of the seed the reducing sugars are totally absent, the pentoses disappear even a little earlier. In the building of the oil the first step is the formation of free fatty acids. Lower and saturated fatty acids are first formed from carbohydrates, probably by an enzyme. The transformation of the fatty acids into neutral glycerides progresses rapidly through the agency of certain active enzymes present in the seed. The activity of the enzyme is maximum when the rate of storage of

oil is the greatest. The unsaturation of the oil increases much in the latter part of the seed life. In the earlier stages much unsaponifiable matter is present in the oil.

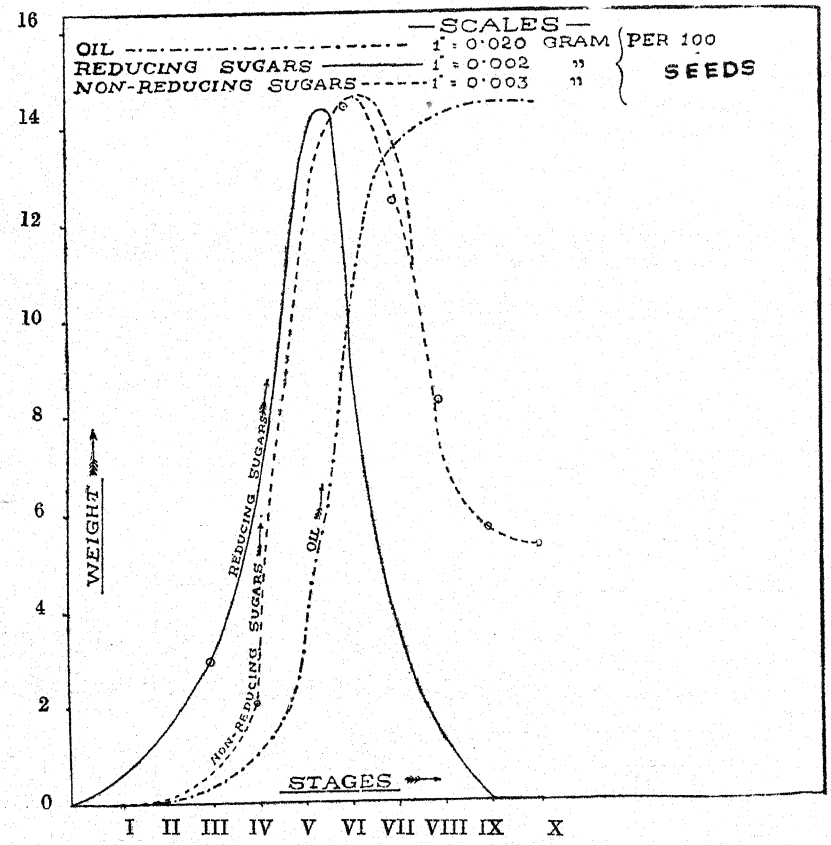


Fig. 1.—Showing relation between oil and sugars.

As regards the unit of carbohydrate which is attacked to form the fatty acids, it seems probable from the results that the lower units of C_6 and C_{12} contribute the maximum amount. Especially the reducing sugars, and hence the C_6 molecule, have very intimate connection in the appearance of the oil in the seed. This fact is vividly brought forward when the graph (Fig. 1) for the oil is compared with those for (1) reducing sugars and (2) non-reducing sugars. In the beginning the reducing sugars rapidly accumulate in the developing seed, attain a maximum and then show a rapid decrease in this amount so that they totally disappear in the resting condition of the seed. This occupies a very short period of the life of the developing seed, *i.e.*, about 1/5th of the whole period.

Till a certain stage of the life of the seed practically very little oil is developed in the seed ; a certain amount of reducing sugars is first accumulated, the development of the seed is sufficiently progressed and then the rate of accumulation of oil becomes enormous. The time required for the major part of the oil to accumulate is comparatively very short. Moreover this period of accumulation of the oil is characterised by the fact that it coincides with the life period of the seed when the reducing-sugar metabolism is the most active. The graphs for the increase of the oil and of the reducing sugars run parallel till the maximum for the reducing sugars is reached. Then the amount of the reducing sugars rapidly decreases and the two graphs are not parallel but only symmetrical about a certain axis in the opposite direction. The maximum of the oil and the minimum of the reducing sugars is reached precisely at the same time of the life of the seed.

To a certain extent the same relation between the amounts of the non-reducing sugars and oil is observed, but only to a limited extent. This is made clear when the graphs for the two, *viz.*, oil and non-reducing sugars, are compared. The rate of increase of accumulation of these sugars is not quite parallel to that of the oil. This storage of non-reducing sugars is not rapid till the accumulation of oil has progressed to a certain extent. So that when the graph for these sugars shows a fall the graph for oil is nearly steady, *i.e.*, a straight line. Perhaps these non-reducing sugars contribute to the formation of insoluble carbohydrates and proteins (Tables IX and XI).

All these facts taken together lead to the following conclusions :—

(1) Fatty acids or fats are formed from carbohydrates in the seed.

(2) The change of carbohydrate to fat takes place in the seed and no fatty substance is brought into the seed.

(3) Finally the unit contributing most to the formation of the fat is the C_6 molecule of glucose, the C_{12} and some pentose C_5 or C_{10} contributing a little.

This would lead us to favour the theory number 2 and a part of 3 mentioned above.

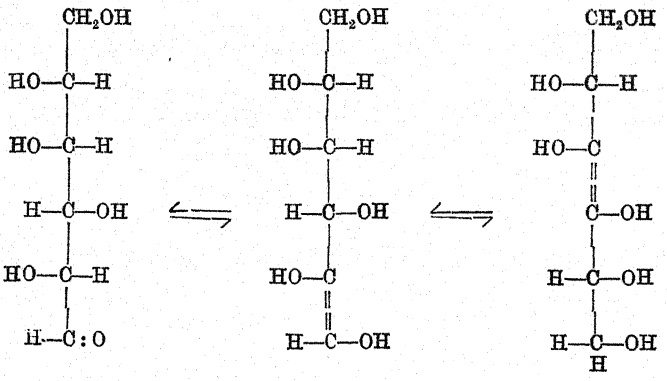
Three glucose molecules may combine together by the process of aldol condensation to form a C_{18} carbon chain. Thus as the hexoses of glucose type predominate, it is most natural that C_{18} acids should be formed in preference to other acids. The presence of pentoses is also important though the percentage is small. They help to explain the formation of other acids. Thus a C_{16} acid may be easily formed by the union of one pentose molecule of C_{10} unit with a hexose molecule of C_6 unit to form an acid C_{16} in length. Similarly it may condense with two hexose molecules to form an acid C_{22}.

Since the condensation would proceed from

$$2\,C_6 \longrightarrow C_{12} + C_6 \longrightarrow C_{18},$$

lower acids will be first formed and this is borne out by the fact that lower acids are formed in the oil in the earlier stages.

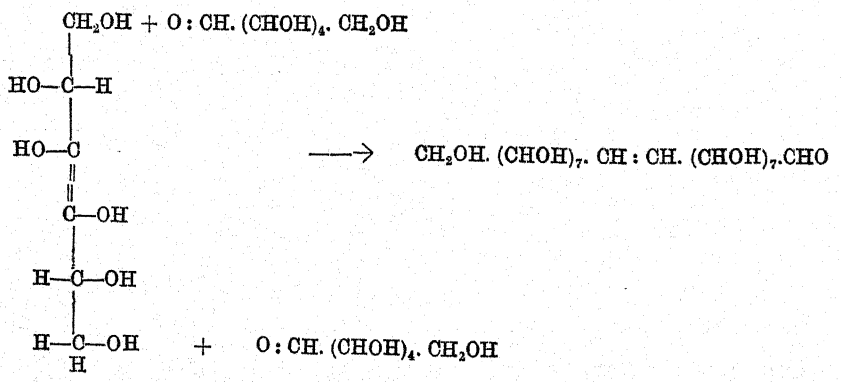
The presence of the double bond in the middle of the chain may be explained in the following way (though no work directly bearing on this suggested theory has been done). It is generally known that the hydroxy group in the δ-position of the glucose molecule is very labile and reactive. Secondly, that the glucose molecule is generally enolised in acid solution in the presence of water, thus



δ-glucose 1 : 2-dienol 3 : 4-dienol

Such enolised molecules may condense together to form a C_{18} chain with the subsequent reduction of the hydroxy group.

Thus,



and then the hydroxy groups may be reduced to hydrogen atoms. Or a 3 : 4-dienol and a 1 : 2-dienol may condense with a dextro-glucose to form linolic acid, the double bond being formed in the 9 : 10 and 13 : 14 positions,

the percentage of the linolic acid formed depending upon the equilibrium percentage of the three molecules.

II. Composition of the niger seed at various stages and the inferences drawn therefrom.

The experiments extended over two years. A plot of 10 *gunthas* was reserved each year and sown with the niger seed in the second week of July. The plants flower by the middle of September, profuse flowering taking place in the first week of October.

General description of the plant and flower.

Plant.—The plant is generally 3 to 5 feet high and has a varying number of flowers ranging from 15 to 40. All the flowers of the same plants do not appear at the same time.

Flower.—The flower is a composite type. It has either 8 or 12 (rarely 9 to 13) stray florets. They form the external whorl of petals of the flowers. The petals of a young flower are yellow in colour, which become golden yellow and finally brown as the age of the flower advances. Inside the big petals there are 3 whorls of florets. In each flower there are about 30 to 34 florets and hence an equal number of ovaries. Out of these about 20 ovaries form well developed seeds. In some flowers the number of florets is 40 to 44 and these flowers give a larger number of well developed seeds.

From the time of opening the flower requires about 40 to 45 days to form a fully developed seed mature and ready for the resting stage.

For experimental work ten stages were marked out, each stage taking about 4 to 5 days to pass from one to another.

Table I.

The number of days required to pass from one stage to another.

Stage	Days after opening
1	0
2	5
3	13 to 15
4	21 to 23
5	26 to 28
6	30 to 32
7	35 to 37
8	41 to 43
9	45 to 47
10 resting	80 onwards

Seed.—In the first stage of the flower it opens out. The petals are yellow in colour, the red tinge is not vivid. As the days advance the red tinge goes on increasing and the petals become golden yellow in colour. It takes about 5 days for the

flower to pass from one stage to another. In the first three stages the seed consists of a white envelope containing an aqueous milk-like fluid, the solid material makes its appearance in the latter period of the third stage. In the fourth stage the coat of the seed begins to assume a violet colour starting from the stem end of the seed which goes on spreading over the whole of the seed and finally deepens and ultimately becomes black. In the fifth stage the outermost whorls have seeds with a violet colour. From the sixth stage onwards the seed presents itself in the form of a black glistening coat having the same appearance (or a little more glistening perhaps) as that of the final stage. In the final resting stage the seed is either glistening black or brown. Up to the fourth stage the coat of the seed cannot be separated from the inner material, which till then is a milky fluid. In the latter part of the fourth stage the cotyledons begin to form as a soft, white material easily crushed and separated into two parts. These soon develop into a solid material and finally assume the form of the two cotyledons of the resting seed. Up to the sixth stage this part is very soft.

Sampling of the seed for analysis.

As already stated the flowers were labelled on the day of their opening. These labelled flowers were sorted out on the day on which they were judged to have reached the stage desired. Such flowers of similar appearance were collected in the required stage and from these flowers the seeds of the two or three external whorls were separated from the florets, cleaned and then taken for analysis and further work. In the first three stages it was impossible to collect a sufficient sample for analysis and ether extraction and hence only a qualitative study of these stages was possible.

Composition of the seed.

General.—The composition of the seed was determined in the usual way [A. O. A. C., 1921] for (*a*) moisture, (*b*) sugars reducing and non-reducing, (*c*) albuminoids, (*d*) cellulose, (*e*) ether extract, and (*f*) ash.

It was found that the results of the two years were in very good agreement as may be seen from the following table :—

TABLE II.

Some of the components of the seed in some stages.

Stage	IV		VI		VIII	
Year	1929	1930	1929	1930	1929	1930
Per cent. moisture . .	66·8	68·0	49·7	52·0	16·4	16·6
„ reducing sugar on dry seed	5·10	5·02	2·85	3·05	0·30	0·15
„ ether extract on dry seed	7·4	7·9	27·2	28·0	40·1	40·5

Weight of the seed at various stages of formation and moisture percentage.

As already indicated flowers were labelled on the day of their opening, and from this the stage of the respective flowers was determined. From such flowers the seeds of the two external whorls were chosen; and out of a large amount of cleaned seed an average sample of not less than 500 seeds was taken. After weighing the measured number of seeds the same sample was kept for moisture determination in a water-oven. When the seeds were dry their weight was taken.

TABLE III.

Weight of the seed in the wet condition and the moisture percentage at the various stages of formation.

Stage	3	4	5	6	7	8	9
Wt. of 100 seeds wet . . .	0·300	0·387	0·465	0·642	0 570	0·419	0·378
Wt. of 100 seeds dry . . .	0·065	0·124	0·210	0·331	0·349	0·342	0·339
Per cent. moisture . . .	80·3	68·05	54·9	49·7	38·6	16·6	9·4

Thus it is evident that till the end of the 6th stage the total weight rapidly increases and then it shows a diminution in weight. Still the weight of the dry matter of the seed is maximum only in the 7th stage.

The proteins of the seed.—The seed which was used for moisture determinations was used for the determination of nitrogen percentage of the seed. This was done by the Kjeldahl-Gunning method (A. O. A. C.). The proteins were calculated by multiplying the figure for nitrogen by 6·25. It is found that the proteins make their appearance at the end of the 3rd stage.

TABLE IV.

Weight of proteins and their percentage at various stages.

Stage	3	4	5	6	7	8	9
Wt. of 100 dry seeds . . .	0·065	0·124	0·210	0·331	0·349	0·342	0·336
Wt. of proteins in 100 seeds (N × 6·25)	..	0·007	0·026	0·055	0·070	0·075	0·073
Percentage on dry seed . .	..	5·45	11·2	17·5	20·6	21·93	21·8

The maximum rate of increase is in the 6th and 7th stages of development. The maximum amount of proteid matter is accumulated in the 8th stage, then a slight decrease in the total absolute amount of proteids is seen.

Ash.—The ash was determined by burning a weighed amount of the seed in the 6th, 8th and the 10th stages only.

TABLE V.

The ash in seed at various stages.

Stage	6	8	10
Wt. of ash of 100 seeds	0·012	0·0168	0·0186
Percentage on dry matter	3·6	4·9	5·4

Ether extract.—The ether extract represents roughly the oil present in the seed together with the unsaponifiable matter soluble in ether like the higher alcohols or the colouring matter.

For this determination the flowers collected were sorted and the seeds from the external two whorls were cleaned out and crushed in a clean mortar and a uniform sample prepared. The ground samples of seeds were then extracted with

petroleum ether, and the oil obtained from the ethereal solution by evaporation of the solvent.

The use of petroleum ether was found to be more convenient as ethyl ether extracted much more colouring matter, water and some reducing substances like sugar along with the water. Thus the figures for the reducing sugars of the samples of seed after extraction with ethyl ether are much lower than those of the same samples before extraction.

TABLE VI.

Amount of reducing sugars (as glucose) as determined by Fehling's solution before and after extraction with ethyl ether.

Stage	3	4	5
Per cent. reducing sugars of extracted seed . . .	5·2	4·8	2·04
Unextracted	6·1	5·7	2·35

All these little difficulties introduce some errors in the further examination of the oil; and hence the use of petroleum ether.

Twenty to twenty-five grams of the cleaned seed in the various stages were prepared into a uniform sample and extracted with petroleum ether and the percentage of the oil determined from the weights of the ether extract and the weight of the seed taken.

TABLE VII.

The weight of oil in seed at various stages.

Stage	3	4	5	6	7	8	9
Weight of 100 dry seeds	0·065	0·124	0·210	0·331	0·349	0·342	0·336
Weight of ether extract	0·00	0·009	0·048	0·0876	0·136	0·143	0·145
Percentage on dry seed	0·0	7·4	17·5	28·0	38·7	40·5	42·9

It will be seen from the above Table and Figure 1 that the oil-percentage increases rapidly within a comparatively very short period of the development of the seed. From the 5th to the 7th stage 21·2 per cent. of the oil, *i.e.*, half of the total amount is brought in the seed. Up to the 5th stage the seed is accumulating

much reducing sugars and the development of the seed progresses when a rapid accumulation of oil is visible.

Carbohydrates.—The carbohydrates of the seed may be classified as follows :—

(1) Water-soluble—

(*a*) Reducing sugars such as glucose.

(*b*) Non-reducing sugars which are hydrolysed by heating with acids, *e.g.* sucrose.

(2) Those insoluble in water but soluble in acids and alkalis. These would be of dextrin or starch type, and are determined indirectly by differences.

(3) The water, alkali and acid-insoluble portion of the seed *minus* the ash. This is designated as " fibre ".

The determinations were done in the usual order, but for the convenience of discussion and comparison the fibre and other carbohydrates are taken first.

Woody fibre.—The woody fibre was determined as usual by digesting a crushed sample of the seed with 5 per cent. alkali, 5 per cent. sulphuric acid, and washing with alcohol and ether. The amount of crude fibre matter insoluble in all the above reagents after boiling for half an hour with each was taken to represent the fibre present in the seed.

TABLE VIII.

The weight of woody fibre in 100 seeds at various stages.

Stage	3	4	5	6	7	8	9
Wt. of 100 dry seed . . .	0·065	0·124	0·210	0·331	0·349	0·342	0·336
Wt. of woody fibre . . .	..	0·040	0·042	0·048	0·050	0·0502	0·0504
Percentage of woody fibre . .	..	32·3	20·0	14·5	14·4	14·8	15·0

Other carbohydrates.—The other carbohydrates were calculated from the various determinations and the weight of the seed in all the stages. This is obtained by subtracting the weights of water, reducing sugar, non-reducing sugar, protein matter, woody fibre, ash and oil from the actual weight of the seed. The following table gives a rough idea of the amount of these carbohydrates thus obtained in the various stages of formation.

TABLE IX.

Approximate weight of the carbohydrates in seeds.

Stage	4	5	6	7	8	9	10
Wt. of 100 dry seeds . . .	0·124	0·210	0·331	0·349	0·342	0·336	0·336
Wt. of carbohydrates in 100 seeds	0·050	0·062	0·077	0·061	0·0432	0·0426	0·0420

Reducing and non-reducing sugars.—Ten grams of fresh seed were crushed in a porcelain mortar and extracted with water. The extract was filtered and then defecated with the least amount of basic lead acetate solution and then made up to 125 c.c. After removing the excess of lead from 50 c.c. of the extract by means of solid sodium phosphate, the reducing sugars were estimated in the usual way [A. O. A. C., 1921, Arts. 24 and 26] by means of Fehling's solution both gravimetrically and volumetrically and the results calculated as glucose. From another 50 c.c. of the same extract non-reducing sugars were estimated by hydrolysing with hydrochloric acid.

TABLE X.

Amount of glucose in 100 seeds in the various stages.

Stage	3	4	5	6	7	8	9
Wt. of 100 dry seeds . . .	0·065	0·124	0·210	0·313	0·349	0·342	0·336
Wt. of reducing sugars in 100 seeds	0·003	0·006	0·013	0·009	0·004	0·001	0·000
Percentage of reducing sugars on dry seed	4·61	5·02	5·72	2·85	1·04	0·30	0·00

The results are calculated on oven-dry basis. As will be shown later on there are present pentose sugars in the seed in the earlier stages and the results are calculated above as glucose, the definite nature of the other type of sugar not being fully known. In the first two stages which were analysed qualitatively the reducing sugars were found to be present in appreciable quantities. It appears that the reducing sugars make their appearance as soon as the seed begins to develop. The

amount of the same goes on increasing to the middle part of the 6th stage and then decreases rapidly. This has a very important correlation with the amount of oil percentage as will be seen on comparing the two graphs (Fig. 1).

Non-reducing sugars.—50 c.c. from the original extract after defecation with lead acetate were hydrolysed with hydrochloric acid, which brought about the inversion of the non-reducing sugars. The amount of reducing sugars thus formed was estimated by titrating with standard Fehling's solution as usual. The results are given as sucrose.

TABLE XI.

Amount of non-reducing sugars as sucrose in 100 seeds in the various stages.

Stage	3	4	5	6	7	8	9
Weight of 100 dry seeds . .	0·065	0·124	0·210	0·313	0·349	0·342	0·336
Weight of non-reducing sugars as sucrose in 100 seeds	0·0007	0·0032	0·0165	0·0218	0·0189	0·0126	0·0087
Percentage of reducing sugars on dry seed	1·1	2·6	7·1	6·6	5·4	3·7	2·65

The graph for this shows a gradual increase in the amount of these sugars and then a decrease in the latter part of the life of the seed. Perhaps this rise and decrease is related to the formation of the other carbohydrates and protein matter which is accumulating in the developing seed.

Relation of fat to carbohydrates.

It is a very interesting thing to note the relation between the absolute amounts of oil and the sugars present in the seed, thus

TABLE XII.

Weights of sugars and oil in 100 seeds.

Stage	3	4	5	6	7	8	9
Weight of reducing sugars . .	0·003	0·0062	0·013	0·0093	0·004	0·001	0·000
Weight of oil	..	0·029	0·0725	0·141	0·145	0·147	0·147
Percentage of reducing sugars .	4·6	5·02	5·72	2·85	1·04	0·3	0·0
Percentage of oil . . .	..	7·4	17·5	28·0	38·7	40·5	42·9

The graphs (Fig. 1) of these two are very striking in that the amount of reducing sugars goes on increasing up to the end of the 5th stage, so that the graph is rising

but as soon as the 6th stage is reached the amount of reducing sugars begins to decrease rapidly so that the graph shows a steep descent, in fact it reaches the line of origin even in the 7th stage of the life of the developing seed. The very reverse is the case with the amount of oil present in the seed. The graph for oil is comparatively a flat curve in the first four stages, in fact even to the middle of the 5th stage, but it soon begins to rise so rapidly that the maximum is soon reached and then the graph assumes the form of a straight line, as the amount of oil remains constant. This seems to lead to the conclusion that probably it is the smaller unit of C_6 or C_5 molecule which contributes most for the formation of the oil in the seed. The non-reducing sugars show more relation with the formation of other carbohydrates and proteids in the seed. Hence the C_{12} unit seems to contribute more to the other food material than to the oil.

The nature of the carbohydrates present in the plant.

Before accepting as a general rule that fats are synthesised from carbohydrates, the fatty acids being the first step towards the building of neutral glycerides, we have to explain a few points.

(1). What is the probable nature of the carbohydrates that are present in the plant in all the stages of seed development ?

(2). Is no other substance excepting the sugar of glucose type brought in to the seed, for example an aldehyde with a straight chain of carbon atoms ?

(3). Are all the transformations carried out in the seed or are they going on in the leaf—the main centre of photo-synthetic activities—and the products of transformation, for example fatty acid, are then brought into the seed ?

As regards the last question Priestley says that fat-metabolism probably starts from carbohydrates and takes place even in the dark, if a liberal supply of carbohydrates be given. He also calls attention to the fact that apparently fat synthesis can only take place in the cell that is going to use the fat or store it, for there are fatty substances present in the transpiration steam. But an interesting substance has been isolated by Curtious and Franzen [1914] by distillation with steam of macerated leaves. After the removal of the acids in the distillate they were able to isolate a-B-hexelene-aldehyde, $CH_3.(CH_2)_2.CH:CH.CHO$. This aldehyde, they state, gives many reactions of formaldehyde. If there be any such substance being brought into the seed in quantity, then it is very likely that such a substance is very easily transformed into a straight chain of fatty acids by the well-known aldol condensation. Thus $CH_3.(CH_2)_2.CH:CH.CHO + HCH_2.(CH_2)_2.CH:CH.CHO$, two aldehyde molecules condense to form $CH_3.(CH_2)_2.CH:CH.CH(OH).CH_2(CH_2)_2.CH:CH.CHO$; this with two more molecules would give a C_{20} aldehyde which then by B-oxidation may give C_{18} acid.

With a view to ascertain this last point a set of experiments was arranged. Aldehydes, if present in the leaves or stem through which the transpiration stream should be flowing to reach the flower, can be detected in a distillate of the same. Some ten plants of average growth and having bunches of flowers were cut down. The flowers and the other parts of the plants were separated; and the two separated portions were then steam-distilled and the distillates collected.

Both the distillates were found to be a little acidic and were neutralised with $N/10$ potassium hydroxide. The neutralised distillates were then extracted with ether. 500 grams of the plant gave only 0·1 gram of a brownish substance. The flowers gave a little less of a similar substance. This substance gives a faint reaction of aldehyde with magenta solution. The quantity and the intensity of the reaction with the magenta solution shows that no volatile aldehyde is being brought in to the seed. As will be shown later on there are present in the seed and plants some pentoses or pentosans, which may yield a little of furfurol if distilled with steam.

Another set of samples of plants and flowers were distilled with steam with previous addition of hydrochloric acid. It was found that the plant portion nearly in all stages gave a brownish viscous material in the distillate. This seems to be an aldehyde, since (*a*) it gives a red colour with decolourised magenta solution and (*b*) a blue-black precipitate with phloroglucinol in hydrochloric acid solution (Holzstoff's reagent). This must be due to pentosans present.

Similarly the seeds in the earlier stages (*viz.*, up to the 7th stage) give the same substance when steam-distilled after the addition of hydrochloric acid. In the last three stages no such substance is obtained unless the distillation is very prolonged. This substance gives a blue-black precipitate with phloroglucinol (Holzstoff's reagent) and also a derivative with phenyl-hydrazine-hydrochloride which melts in the vicinity of 190°C. This derivative could not be identified as the quantity was not sufficient to recrystalise. Thus it is evident that the seed and the plant contain pentoses or pentosans which when distilled with hydrochloric acid yield furfurol. But the weight of the blue-black precipitate with phloroglucinol is enough to account for only a small portion of the reducing sugars present in the seed at the various stages. This shows clearly that along with the hexoses some pentoses are also present in the seed in the earlier stages of the life, which finally disappear and are absent in the resting condition of the seed.

Another attempt made to study the product brought into the seed with transpiration stream was the following :—

The stems of plants which carried bunches of flowers in various stages of formation were cut just below the stalk of the bunch and the open end of the plant side of the stem was connected to a collecting glass tube by means of a short rubber

tube. To this collecting tube was another outlet which was connected to an aspirator, so that a slight suction could be applied to the stem of the plant, to help the transpiration stream. Many such tubes were kept attached to a number of plants for two days. The collecting tubes before being attached to the plants were moistened with a little toluene to stop any undesirable change being brought about in the sap collected. Each plant gave about $\frac{1}{2}$ c.c. of sap in two days when the plants were well watered.

Such sap collected was then tested for carbohydrates and fatty material. It was observed that the sap gives tests for carbohydrates with Mollsch's reagent. The sap gives a slight coloration with magenta solution. But it gives no indication of fats or fatty substances.

Of course the means used are a little unnatural and it may be argued that the sap collected in this way may be an improvised substance brought by the plant to heal the wound. But still the experiment is not without its significance. For this is the general method employed to show the root pressure of plants, which shows that the wound is not easily healed and the transpiration stream goes on at least for some time after the wound is made.

Thus all this points to the conclusion that the only material brought in the seed is of the carbohydrate nature, of course leaving apart the nitrogenous and inorganic materials. Secondly, at least in the earlier stages of development some pentose sugar is brought in the seed which rapidly vanishes and is totally absent in the later stages of seed life. So also other sugars of the reducing type like glucose are brought in the seed and then transformed either into fatty acid, other carbohydrates of dextrine type, or albuminoids, most probably to a greater extent into fatty acids. The presence of pentose sugars explains some important aspects of the theory to be propounded for the fat metabolism. Thus it is easier to explain the formation of C_{16} acids as well as the C_{18} acids. For it is possible that one hexose and two pentose molecules may condense together to form a C_{16} chain.

It appears also that C_6 (or C_5) is more responsible for fatty acid formation than the bigger molecule—C_{18} of starch.

Starch as such was not detected in any of the stages of formation. A few seeds were crushed with a little cold water and filtered through cloth. The filtrate when tested with an iodine solution gave no tests for starch. Similarly thin sections of the seed in all stages were examined under the microscope for starch, yet in no stage could starch be detected. Only in the later stages a brown coloration was visible when the slide was stained with iodine as in a sample of dextrine. Similarly when the cake of seed left over after extraction with ether and alcohol in the later stages was extracted with hot water a brown coloration was visible with iodine solution.

This points out that starch is practically absent in the seed in any stage of formation or in the resting condition.

III. Some important constants of the niger seed oil at various stages.

The petroleum ether extract was used for the determination of chemical and physical constants. The oil in the earlier stages is pale green in colour, and in the very early stages, namely, between the 3rd and the 4th stages, deposits some solid fat or fatty acids even at ordinary temperatures. The melting point is also higher in the earlier stages.

Table XIII.

Melting point of the oil.

Stage of seed life	3 to 4	6 to 7	9 to 10
Oil melts at °C.	2 to 3	—1 to 0·0	—7 to —8·5

So also the butyro refractometer reading is lower in the earlier stages of formation.

Table XIV.

The scale reading of the oil at various stages at 28°C.

Stage	3	4 to 5	7 to 8	9	10
Scale reading at 28°C. . .	59	62·4	65	67	67·5
Corresponding N_d . . .	1·4652	1·4674	1·4691	1·4704	1·4707

Thus it is evident that more saturated acids of oil are present in the earlier stages, because (*i*) the melting point and (*ii*) the refractive index are higher in the earlier stages than in the later stages.

The chemical constants determined were the free acidity, the saponification value, iodine value, the unsaponifiable matter and the acetyl value in some cases.

The free acidity and saponification value.—It was found that in the earlier stages the extract contains much free acidity. The apparent saponification value

is lower in the beginning, but really the equivalent of the acids is higher and hence the saponification value is also higher. The oil contains much unsaponifiable matter.

2·5 to 3 grams of the ether extract were weighed out into a 300 c.c. flask and mixed with 25 c.c. of neutral alcohol and the free acidity determined by titrating with $N/10$ KOH in 50 per cent. alcohol solution using phenolphthalein as indicator. To the same were then added 20 c.c. of $N/2$ alcoholic KOH and the oil saponified by refluxing the solution for half an hour in the usual way. After titrating the excess of alkali with hydrochloric acid ($N/2$), the saponification value was calculated. From this saponified sample of oil the alcohol was removed after making the soap solution strongly alkaline with a little KOH—by evaporation on water bath. The soaps were then dissolved in 150 c.c. of water. This soap solution was then extracted with petroleum ether. From the soap solution after extraction the free fatty acids were liberated by an excess of hydrochloric acid and the free acids extracted with ether recovered and weighed. This gave the percentage of the fatty acids. The equivalent of these free acids was then determined by titrating with standard KOH solution.

TABLE XV.

Free acidity as milligrams of KOH per gram of oil.

Stage	3	5	7	9	10
KOH	110	41·5	14·6	8·8	4·6

TABLE XVI.

The apparent saponification value in milligrams of KOH per gram of oil.

Stage	3	5	7	9	10
Sap. value	160·0	180·2	192·7	193·4	194·8

Thus the free acidity is very high in the earlier stages, the ratio of the free acidity to the total saponification value is given in the following table :—

TABLE XVII.

Ratio of free acidity to saponification value multiplied by 100.

Stage	3	5	7	9	10
Ratio F. A./S. V. multiplied by 100	68·7	23·3	7·6	4·5	2·3

As regards the low saponification values they are due to the large amount of unsaponifiable matter present in the earlier stages of seed development. Thus the saponification value is actually higher in the earlier stages if the free fatty acid percentage is taken into consideration. This is also borne out by the equivalents of the free acids as obtained from the oil after removal of the unsaponifiable matter. The following table gives the free fatty acid percentage determined as indicated above. It also gives the equivalent of the free fatty acids, (1) when determined by titrating the acids in alcoholic solution, with KOH ; and (2) the calculated equivalent of the same from the percentage of acids.

TABLE XVIII.

Fatty acid percentage and molecular equivalents of the same.

Stage	3	5	7	9	10
Percentage fatty acids . .	71·25	85·24	92·96	94·0	94·2 to 94·7
* Equivalent in mgs. KOH per gram acid calculated	224·6	211·18	209·0	208·0	208·0
Actually obtained . . .	225·1	212·00	210·4	208·2	208·0

* The calculated equivalent is equal to $\frac{\text{Saponification value} \times 100}{\text{Percentage of fatty acids}}$ as calculated from the apparent saponification value of the ether extract. Thus in the third stage equivalent of the acids in mgms. KOH is equal to $\left(\frac{160 \text{ mgms. KOH} \times 100}{71{\cdot}25}\right)$—the saponification value divided by the percentage of the fatty acids—224·6 mgms. KOH.

The figures for equivalents of the free fatty acids as calculated are in very good agreement with those that were actually determined. This is evident from

the above Table. Hence it appears that the lower fatty acids are synthesised in the earlier stages and as the age advances the higher fatty acids are formed.

Iodine value of oil and fatty acids.—The iodine value was determined by weighing about 0·15 to 0·2 gram of the oil or the acids, as obtained after removal of unsaponifiable matter, into a dry bottle with good stopper. The substance was dissolved in 15 c. c. of carbon tetrachloride, and 10 c. c. of a *N* bromine solution in carbon tetrachloride were added and the stoppered bottle kept in ice water in a dark cupboard to protect the reacting mixture from light as far as possible. After two hours 10 c.c. of a saturated solution of potassium iodide were added to the bottle, the whole shaken and the free iodine thus liberated was titrated with a standard sodium thiosulphate solution. In every set a blank was kept and from the reading for the blank and the substance the amount of bromine or the equivalent iodine absorbed was calculated. The oil and acids were kept at the same time and under the same conditions to avoid any error of the method.

TABLE XIX.

Iodine value of the oil and fatty acids obtained from the oil after removal of unsaponifiable matter.

Stage	3	5	7	8-9	10
Iodine value of oil . .	89·5	94·35	102·15	112·14	126·2
Iodine value of acids . .	87·4	92·1	105·4	130·80	133·4

Thus the iodine value is low in the earlier stages of formation and increases as seed develops, showing that saturated acids are first formed and then the unsaturated fatty acids are developed. Perhaps it is possible that acids with hydroxy groups are first formed and the ethenoid linkage generated by removal of a water molecule; thus:—

$X.CH(OH).CH_2.X' \longrightarrow X.CH : CH.X' + H_2O$, or,

two different molecules may combine to form a bigger molecule,

$X.CH_2.OH + H.CH.OH.X' \longrightarrow X.CH : CH.X' + H_2O$

This is a very likely course of synthesis for as has been shown already the lower acids do make up their appearance in the earlier stages of formation, and because the acetyl value of the oil in the earlier stages is also higher, showing that more hydroxy groups are present in the earlier stages. The acetyl value as milligrams of KOH required to neutralise the acetic acid from the acetylated product are given below.

TABLE XX.

Acetyl value at different stages.

Stage	4	7	10
Acetyl value	62·4	51·4	23·2

This cannot be, of course, taken as a definite proof of the presence of hydroxy-acids in the oil for it is possible that if there is any free glycerine or some mono- or di-glycerides in the oil then these glycerides may also form acetic esters when boiled with acetic anhydrides.

The iodine value of the oil as given above is only apparent, for the unsaponifiable matter which is present was also observed to absorb bromine (or iodine) which means it has some iodine value so that the real iodine value of the fatty acids is still lower than that deduced from that of oil in the earlier stages. The iodine value of free acids ought to be higher than the iodine value of the oil, if the iodine value of the unsaponifiable matter is not high. But since the iodine value of the unsaponifiable matter is nearly of the same order as of the oil and as the percentage of unsaponifiable matter is high in the earlier stages the apparent iodine value of the oil in the 3rd stage is higher than that of acids (*vide* Table XIX) whereas it ought to have been lower. So that it appears that the real unsaturation of the acids is still lower in the earlier stages than is indicated. It is most likely that oleic acid is first formed and linolic acid is formed later on.

Reichert Meissl value.—This value which indicates the percentage of volatile fatty acids, *i.e.*, of low molecular weights is also greater in the earlier stages.

TABLE XXI.

Reichert Meissl value in c.c. of KOH (N/10).

Stage	3	6	10
R. M. Value	2·36	2·42	1·11

All these results show that in the earlier stages of the seed development lower saturated acids are formed and the unsaturated acids and higher acids are formed in the latter period of the life-history of the seed.

Unsaponifiable matter.—The large percentage of unsaponifiable matter in the earlier stages seems to be a viscous material which gives an intense green colour with the Wellman's reagent.

IV.—ENZYMES CONNECTED WITH THE FORMATION OF OIL IN THE NIGER SEED.

It is shown in the previous chapter that only sugars are brought into the seed by transpiration; and these sugars are subsequently transformed into fatty acids.

It is highly probable that such a change is brought about by an enzyme.

In order to ascertain whether this is so, laboratory experiments were arranged. The results obtained give enough indication that the sugars are changed into acids by an enzyme.

An extract of the seed in the various stages was prepared by crushing and grinding the cleaned seeds with ten times its weight of distilled water three successive times. A set of six experiments was kept for each stage.

(*a*) 50 c.c. of extract and 100 c.c. distilled water;

(*b*) 50 c.c. of extract and 100 c.c. of one per cent. glucose and one per cent. levulose solution;

(*c*) 50 c.c. of extract boiled for half an hour and 100 c.c. of the sugars in solutions;

(*d*) 50 c.c. distilled water and 100 c.c. of the sugars in solutions.

All the four solutions were kept in flasks and plugged with cotton wool and shaken from time to time. After the fixed interval of time an aliquot portion of the mixture was pipetted out and titrated with *N*/10 KOH solution using phenolphthalein as indicator. The following Table gives the number of c.c. of *N*/10 KOH required to neutralise 10 c.c. of the reacting mixture.

TABLE XXII.

Volume in c.c. of N/10 KOH required for 10 c.c. of solution.

Stage 4th to 5th

Time in hours	0 hr.	4 hrs.	10 hrs.	24 hrs.
Set (*a*)	0·5	0·6	0·8	0·85
(*b*)	0·5	1·1	1·35	2·18
(*c*)	0·6	..	No change	
(*d*)	less than 0·1	..	No change	

Stage 7th to 8th

Set (*a*)	0·3	0·45	0·5	0·5
(*b*)	0·3	0·55	0·65	0·65
(*c*)	0·3	..	No change	
(*d*)	less than 0·1	..	No change	

Thus there is a much more increase in the acid value when the extract is treated with extra amount of sugars than the set to which no sugar is added. The one which is boiled before the experiment does not show any rise in acid value. This indicates a possibility of bringing about the change of carbohydrate into some acid by an agent present in the developing seed.

The esterase of the seed.

It has long been known that the seeds, especially oil seeds, contain a certain enzyme—esterase—which has the property of synthesising glycerides and esters of fatty acids with glycerine and other alcohols. Also it is an accepted fact that the nature of the reaction is reversible in character. Fokin [1906] had concluded that the hydrolysis of oils by seed ferments is not quite reversible though the hydrolysis went further if the glycerol, one of the products of hydrolysis, was removed. But still later work has confirmed the reversible nature of the seed enzyme, and that the glycerides may be synthesised by means of both animal and vegetable ferments. Welter [1911] using 100 parts of fatty acids, 20 parts pure glycerine and 10 parts of castor seed ferment has shown that the acid value of the mixture diminishes during the first two days and the values obtained indicated that the following percentages of fatty acids combined in the case of the acids named :—

Palmkernel oil acids	30 per cent.
Coconut „ „	21 „
Groundnut „ „	19 „
Oleic acid	26 „

No reaction occurs if the glycerol is omitted so that the diminution in the acid value is not due to anhydride formation. Taking into consideration the fact that the free fatty acids are the first step in the formation of oil in plants, it is natural that such an enzyme which would bring about the synthesis of neutral glycerides from fatty acids and glycerine should be present in the developing seed. Simple fatty acids and glycerine appear to remain unchanged for a very long time.

It has been suggested that probably the same enzyme—lipase—concerned in the hydrolysis of fat may bring about their synthesis, or a different enzyme may be involved in each process.

Ivanow [Harvey, 1929] has shown that the seed have the necessary catalyst for both the synthesis and the hydrolysis of fats. He used poppy seed which are normally rich in oil containing saturated fatty acids, and flax seed which are rich in oil containing unsaturated fatty acids. The enzyme of each kind was extracted with glycerine. To the glycerine extracts of poppy seed free oleic acid was added. In three months the acid number fell from 44·6 to 34·84. When oleic acid was added to the flax seed extract the acid number fell from 80·04 to 51·4 in three months and 16 days. There was no decrease in the acid number of blanks which

had been boiled to stop the action of the enzyme. Evidently there is esterification of the fatty acids and glycerine by some substance present in the seed—an enzyme or catalase—soluble in glycerine. Preliminary work on the composition of the niger seed had shown that the first step in the formation of oil in the niger seed was the appearance of free fatty acids. Hence it was thought that there must be an enzyme present in the seed which would transform these fatty acids into neutral glycerides. Secondly if there be any such enzyme present in the developing seed its activity may be different in the various stages of the seed development. For as already indicated the ratio of free fatty acids to the oil present is different in the different stages, and also the rate of increase in the oil percentage is also different in the various stages.

It was therefore decided to investigate (1) whether there is any such enzyme present in the seed, (2) whether its activity is different in different stages, (3) whether the same enzyme is able to bring about esterification as well as hydrolysis of oil, if a little excess of oil is added.

The results seem to point out that there is an enzyme present in the seed which is capable of bringing about the esterification of free fatty acids and glycerine. The activity of the enzyme is different in the various stages of development, and is greatest when the rate of increase in the oil percentage of the seed is greatest, *i.e.*, in the 5th and the 6th stages.

The enzyme does not seem to be capable of hydrolysing the oil. Some hold the view that there is only one enzyme responsible for both the synthesis and hydrolysis of oil; and the percentage of water in the seed determines whether the synthesis or hydrolysis of the oil will predominate. This view in the light of the above experiment does not seem to be very exact.

The stage of the seed from which the extract is prepared seems to be the more probable controlling factor. The addition of water or oil seems to influence the reaction in the same way as an excess of one of the reaction products would influence any mono-molecular reaction. Thus a fair excess of oil is not sufficient to stop all the esterification of fatty acids, though it seriously hinders the rate of the reaction, if the extract has been prepared from the seed in the 5th or the 6th stage, when the oil accumulation is most rapid. While the same proportional amount of oil when added to an extract in the later stages, say 9th stage, has a very great retarding influence on the amount of esterification. In fact a slight increase is observed in the acid value of the reaction mixture, when the oil added is only 5 per cent. of the total reacting mixture, if the seed used for the preparation of the extract is from the last stage. The same view is upheld by the fact that in the resting stage the lipase is less active than in the germinating seed when the radical is protruding. The resting stage seed seems to be incapable of

giving any extract which is active in bringing about the esterification of free fatty acids to any great extent.

In short it seems that the nature of the enzyme affecting the fats or fatty acids, changes with the age of the seed from which the extract is obtained. If it is only one enzyme it has the character of an esterase—catalase—in the earlier life-history of the developing seed, while it assumes lipolitic character in the later stages, *viz.*, the germinating stage. If there be two different enzymes in the seed then the esterase predominates in the earlier part of the life of the seed, and the lipase develops in the later stages—the resting and the germinating—when the already stored fatty material is to be used for the benefit of the newly developing plant.

The experimental part.

As is already indicated the seeds were collected in various stages by collecting them from flowers which were labelled on the day of their opening. The seed was separated from the two external whorls and then taken for the preparation of the extracts.

Pure glycerol (strength 98·6 per cent.) was taken for the experiment. The fatty acids used for the experiment were prepared from the niger seed oil by first hydrolising the oil with caustic potash in alcoholic solution, the alcohol removed by evaporation and the fatty acids liberated from the soaps (dissolved in water) by strong hydrochloric acid. The liberated acids extracted with ether and the etherial solution dried over anhydrous magnesium sulphate, the ether removed and the fatty acids recovered, and kept out of contact with air.

The extraction of the enzyme.—Preliminary work showed that it is better to use a mixture of equal parts of glycerine and fatty acids rather than the pure glycerine for the extraction of the enzyme; for it appears that the concentrated glycerine has a dehydrating action on the fresh seed. The mixture of acids and glycerine extracted the enzyme to the same extent.

The seed contains different amounts of water, and therefore the weight of the seed taken for extraction in the various stages was different. It amounted to about 10 grams of dry material per 150 c.c. of the final reacting mixtures. Thus the weights actually taken are given in Table XXIII.

TABLE XXIII.

Stage between	4 and 5	6 and 7	8 and 9	Resting
Wt. in grams of wet seed	20	15	12	11
Wt. of dry seed	9·1	9·4	10·1	10·4

The weighed amount of seed was well crushed and then ground with 20—20 and 10 c.c. of the mixture of glycerine and acids three successive times and the extract filtered through a fine cloth to remove the coarser grains of the seed. The extract carried with it some fine aleurone grains, but it was not thought necessary to remove them. Thus about 500 c.c. of the extract were collected. This was then mixed with 100 c.c. of acids or acids and oil as the case may be. For every stage a set of seven different mixtures was kept.

(1) 50 c.c. extract and 100 c.c. fatty acids.
(2) 50 c.c. extract and 100 c.c. fatty acids, the extract heated to 110°C. for half an hour before mixing.
(3) 50 c.c. of glycerine-acid mixture and 100 c.c. fatty acids and 0·75 c.c. glacial acetic acid (Blank).
(4) 50 c.c. extract and 100 c.c. fatty acids and 0·75 c.c. glacial acetic acid.
(5) 50 c.c. extract heated to 110° C. for half an hour and 100 c.c. fatty acids and 0·75 c.c. glacial acetic acid.
(6) The same as (4) *plus* 10 c.c. water.
(7) The same as (4) *plus* 10 c.c. of oil.

All these sets were kept in sterlized flasks plugged with sterlized cotton wool and kept under the same conditions. The minimum temperature for the period was 25°C. and maximum 29·6°C. in the laboratory where the flasks were kept. The reacting mixtures contained approximately 25 c.c. glycerine and 125 c.c. fatty acids to 10 grms. of dry seed weight.

The addition of glacial acetic acid to the extent of 0·5 per cent. accelerates the esterification; and it was found that larger amounts of acetic acid had not much effect on the rate of reaction, excepting that the maximum or total esterification was a little more than when 0·5 per cent. was used. The addition of this acid seems to accelerate the reaction by creating a higher acid concentration.

The reacting mixture was nearly uniform in the last stages when the water per cent. in the seed was not much, and in earlier stages when there was much moisture or in cases when extra water was added the glycerine showed a tendency to separate from the mixture to some extent and had to be shaken occasionally throughout the experiment.

It was found that by heating the extract to 110°C. for half an hour the activity of the enzyme was destroyed. This is evident from the sets (1) and (2), or, (4) and (5).

The mixture of glycerine and fatty acids which had no extract of seed showed no diminution in the acid value, so that the diminution in the other cases must be due to the esterification brought about by some enzyme present in the seed. Secondly the saponification value of some of the typical sets after the completion of the experiment was determined and found to agree closely with the original acid value.

The determination of the free acidity was done in the following way :—

Five to six grams of the reacting mixture were weighed out, after shaking the reacting mixture thoroughly to ensure a uniform sample. To this were added 25 to 30 c.c. of neutral alcohol and the whole was then titrated with a $N/10$ potassium hydroxide solution using phenolphthalein as indicator. The number of c.c. of KOH required per gram of the reacting mixture are given in the following tables. Unnecessary figures for blanks and other titrations where the change was very little have been omitted to reduce unnecessary lengthiness. Generally the blanks dit not vary much from the zero-reading.

TABLE XXIV.

No. of c.c. of N/10 KOH per gram of the reacting mixture. Stage 4th to 5th.

No. of days	Extract and acids	Extract heated and acids	Blank with 0·5 acetic acid	Extract acids and acetic acid	Extract heated and acetic acid	(4) and 10 c.c. water	(4) and 10 c.c. oil
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	23·64	23·60	24·54	24·44	24·04	23·64	23·62
1	23·02	23·58	23·52	23·50	24·00	23·04	23·08
2	22·72	..	23·22	..	..	22·76	22·78
3	22·68	..	23·07	..	..	22·70	..
6	22·60	..	22·90	..	..	22·63	22·64
9	22·58	..	22·75	..	..	22·59	..
12	22·51	..	22·60	..	..	22·54	22·59
15	22·45	23·54	22·46	23·48	24·00	22·47	..
18	22·36	..	22·34	..	..	22·39	..
21	22·26	..	22·22	..	..	22·36	..
24	22·24	..	22·10	..	..	22·31	..
27	22·21	..	22·10	..	..	22·29	22·42
30	22·19	..	21·94	..	..	22·27	..
33	22·16	..	21·88	..	..	22·26	22·44
36	22·14	23·54	21·86	23·45	23·98	22·26	22·45
(Per cent. esterification $= \frac{a-b}{a} \times 100$.)							
$= 100 \times \frac{1·50}{23·64}$		—	$100 \times \frac{2·63}{24·44}$	—	—	$100 \times \frac{1·38}{23·64}$	$00 \times \frac{1·18}{23·62}$
	$= 6·3$	—	$= 10·8$	—	—	$= 5·8$	$= 5·6$

TABLE XXV.

No. of c.c. of N/10 KOH per gram of the reacting mixture.

Stages 6 to 7

No. of days	Extract and acids	Extract heated and acids	Blank with 0·5 acetic acid	Extract acids and acetic acid	Extract heated and acetic acid	(4) and 10 c.c. water	(4) and 10 c.c. oil
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
0	27·82	26·70	26·80	26·86	27·84	26·56	26·54
1	27·44	..	..	26·48	..	26·34	26·35
2	27·16	..	..	26·18	..	26·12	26·14
3	26·86	26·48	26·80	25·65	27·78	26·00	26·08
6	26·62	..	..	24·92	..	25·62	..
9	25·50	..	..	23·78	..	25·06	25·12
12	24·60	..	..	22·76	..	24·62	..
15	23·68	26·40	26·70	21·46	27·72	24·08	24·24
18	22·80	..	..	21·16	..	23·44	..
21	22·00	..	..	20·00	..	23·32	23·50
24	21·86	..	..	19·72	..	23·02	..
28	21·68	..	..	18·72	..	22·74	22·90
30	21·56	26·36	26·70	18·32	27·72	22·50	22·70
33	21·50	..	..	18·28	..	22·32	..
36	21·48	..	..	18·25	..	22·16	22·42
40	21·46	26·30	26·70	18·23	27·72	22·16	22·44

(Per cent. esterification = $\frac{a - b}{a} \times 100$.)

$=100\times\frac{6·36}{27·82}$	$100\times\frac{0·40}{26·70}$	$100\times\frac{0·10}{26·80}$	$100\times\frac{8·63}{26·86}$	$100\times\frac{0·12}{27·84}$	$100\times\frac{4·4}{26·56}$	$100\times\frac{4·2}{26·54}$
$=22·85$	—	—	$=32·13$	—	$=16·57$	$=16·54$

Tables XXIII & XXIV.—The percentage of esterification brought about by the extract of the seed amounts to 10·8 per cent. when 0·5 per cent. of glacial acetic

acid was added; when this was omitted the esterification seems to proceed to 6·3 per cent. only. The increase is 4·5 per cent. This is more than the acetic acid used. Hence it is evident that the acetic acid seems to act as a catalyst. The addition of 5 per cent. water decreases the rate as well as the extent of esterification. The addition of 5 per cent. oil lowers still more the rate and extent of esterification, but it does not completely stop the reaction taking place. In the 6th or the 7th stage the figures for which are given in Table XXV, the esterase seems to be more active than in any other stage. The total esterification brought about is found to be 22·85 per cent. and 32·13 per cent. when 0·5 per cent. of glacial acetic acid was added. Thus in this case the catalytic effect of the acetic acid is more pronounced than in the previous experiment.

The rate in the case of acid mixtures, *viz.*, (1) and (4), is in the beginning very rapid which then slows down as the days proceed and in the end is nearly constant. The addition of 10 c.c. of oil to the reacting mixture makes the rate of esterification more uniform and the extent of reaction is also lowered.

TABLE XXVI.

No. of c.c. of N/10 KOH per gram of reacting mixture.

Stages 8th-9th

No. of days	Set (1)	Set (2)	Set (3)	Set (4)	Set (5)	Set (6)	Set (7)
0	26·56	26·60	26·50	26·92	26·60	26·36	26·38
2	26·18	..	..	26·36	..	26·08	26·08
9	25·54	..	..	25·62	..	25·50	25·58
21	25·06	..	..	24·42	..	25·32	25·42
30	25·16	..	..	23·58	..	25·30	25·44
36	25·14	26·50	26·30	23·52	26·42	25·30	25·44
(Per cent. esterification = $\frac{a-b}{a} \times 100$.)							
	$=100\times\frac{1·40}{26·56}$	—	—	$100\times\frac{3·40}{26·92}$	—	$100\times\frac{1·06}{26·36}$	$100\times\frac{0·86}{26·38}$
	=5·3	—	—	=14·15	—	=3·85	=3·2

Towards the end of the experiment the activity of the enzyme appears to decrease and the addition of oil shows a slight increase in the acid value, thus showing that the reaction is reversible in nature.

TABLE XXVII.

No. of c.c. of N/10 KOH per gram of reacting mixture.

Resting stage of the seed

No. of days	Set (1)	Set (2)	Set (3)	Set (4)	Set (5)	Set (6)	Set (7)
0	23·10	23·60	23·68	23·80	24·02	21·20	21·34
10	22·18	..	..	22·78	..	20·67	20·80
15	21·98	..	..	22·66	..	20·60	20·80
21	21·90	..	..	22·58	..	20·60	20·82
30	21·92	..	..	22·50	..	20·62	20·82
40	21·92	23·56	23·60	22·50	24·00	20·62	20·82
(Per cent. esterification = $\frac{a-b}{a}\times 100$.)							
	$=100\times\frac{1\cdot2}{23\cdot10}$	—	—	$100\times\frac{1\cdot3}{23\cdot80}$	—	$100\times\frac{0\cdot6}{21\cdot20}$	$100\times\frac{0\cdot52}{21\cdot34}$
	$=5\cdot2$	—	—	$=5\cdot56$	—	$=2\cdot9$	$=2\cdot1$

Thus in this stage the activity is very low, moreover the addition of acetic acid seems to have very little effect on the reaction. In the cases where water and oil have been added the acidity increases in the end, which shows that hydrolysis has started. Perhaps this may be added as a proof that the nature of the enzyme changes in character with the age of the seed. The total esterification brought about in the various stages is given in the following Table.

TABLE XXVIII.

Percentage of esterification brought about.

Stage	4th to 5th	5th to 6th	7th to 8th	Resting
With acetic acid	10·8	32·13	14·15	5·56
Without acetic acid . . .	6·3	22·85	5·3	5·2
7 per cent. water . . .	5·8	16·57	3·85	2·9
7 per cent. oil	5·6	16·54	3·2	2·1

CONCLUSIONS.

It has been shown that the oil is formed in the seed from carbohydrates. Lower saturated acids are formed first and then the higher and unsaturated acids. The change of the carbohydrates to fatty acids is brought about by some enzyme present in the developing seed. The free fatty acids are accumulated and these are then transformed by means of an enzyme—esterase—into neutral glycerides. The activity of this enzyme is different in different stages and is maximum when the oil percentage is increasing very rapidly.

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LEAF-CURL IN *ZINNIA ELEGANS* AT DEHRA DUN.

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(With Plates IX and X)

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I. INTRODUCTION.

The investigations discussed in this note were suggested by Mr. R. N. Parker, Forest Botanist, and planned by Dr. C. F. C. Beeson, Forest Entomologist, under whose supervision they were carried out by the writer in 1930-31, in the Insectary attached to the Branch of Forest Entomology. The main reason for the enquiry was the supposition that it would provide experience which would be of value in studying the suspected transmission by certain insects of the spike-disease of sandal [Dover, 1932], which forms an important part of the present activities of the Branch [Beeson, 1932]. The leaf-curl of garden Zinnias was thought to be so obviously the result of virus infection that the usual preliminaries of diseased patch and leaf-grafting and sap inoculation were not tried, attention being concentrated on transmission experiments with the Aleurodids suggested, by an unusually successful indicator experiment (III, a), as vectors. With the conclusion of these experiments and the identification of the Aleurodid it became evident that the disease under study was similar to the leaf-curl of cotton investigated by Kirkpatrick [1931] in the Sudan. The presence of this important disease in garden Zinnias suggests the need for a more elaborate investigation to be undertaken on the possibility of transmission of the Zinnia disease to cotton.

I am indebted to Dr. C. F. C. Beeson for his interest and practical encouragement, and to Mr. Cedric Dover for helpful suggestions in the preparation of this paper. Mr. Karam Singh Lamba, College of Science, Nagpur, kindly indentified the vector.

II. The disease and its Aleurodid vector.

An acropetal necrosis [Quanjer, 1931] or leaf-curl of garden Zinnias (horticultural varieties of *Zinnia elegans*) is very prevalent in Dehra Dun. The symptoms appear to be identical with those of the leaf-curl of cotton in the Sudan [Kirkpatrick, 1931], and it is noteworthy that *Bemisia gossypiperda* Misra and Lamba, the only Aleurodid in the extensive list of Homopterous vectors of viruses [Smith, 1931], is responsible for the transmission of leaf-curl in *Zinnia elegans*, cotton and other malvaceous plants. A characteristic effect of the disease here discussed is the thickening of the lower surface of the small veins of the leaves, preceded by curling of the leaf-blades. The younger leaves at the growing points are first affected, the infection resulting in derangement of the normal growth processes. Sometimes only a part of a leaf becomes crinkly, the rest remaining unaffected.

Plants may be attacked at any stage, but younger plants are generally more susceptible than older plants, in which the symptoms are often less definite and practically confined to the lateral shoots or a few older leaves. Young diseased plants become dwarfed, but may remain alive for months in that condition, which is preceded by bronzing, shrivelling and falling of the affected leaves. Such plants do not attain much more than a foot in height, while healthy plants grow to a height of four or five feet. Dwarfed flowers of dull colours and partial sterility, are among the effects of the disease. Plate IX illustrates these symptoms. A badly diseased plant, with extremely dwarfed flowers, is shown in Plate X.

During the summer and rains axillary buds appear to be forced into growth, which develop, as the season advances, into stunted shoots with small crinkled leaves frequently massed together in rosette or bunchy form. Under winter conditions the development of the disease is retarded.

The growth period of Zinnias is June-November, leaf-curl infection being most evident between July-September. The maximum incidence of the disease therefore coincides with the period when the vector is most abundant, as observed by Afzal Husain [1930] in the Punjab and by the author in Dehra Dun.

The vector, *Bemisia gossypiperda*, was described by Misra and Lamba [1929] as an important pest of cotton in the Punjab, and has been the subject of further study by Afzal Husain [1930, 1] and Kirkpatrick [1931]. It is a polyphagous species known to occur on more than three dozen plants besides cotton [Afzal Husain, 1930, 1], many of which are listed by Misra and Lamba. *Zinnia elegans*

Plants of *Zinnia elegans*, (A) and healthy (B) with characteristic symptoms of leaf-curl,

has not, however, been previously recorded as a host plant. In Dehra Dun alternate host plants, other than Zinnias, have not yet been observed.

In the Sudan it has been definitely established that *B. gossypiperda* is the vector of leaf-curl of cotton, Kirkpatrick [1931] concluding that it "is not only an extremely efficient transmitter of the leaf-curl, but is in all reasonable probability the only vector of the disease." The results of the present investigation support this conclusion. This Aleurodid, however, is not yet known to transmit leaf-curl to cotton or other host plants in the Punjab [Afzal Husain, 1930,2], and Kirkpatrick's studies [1931] indicate that Asiatic cottons are at least highly resistant to leaf-curl infection.

III. Transmission experiments.

(*a*). *Methods employed.*

Zinnia seeds from various sources were obtained locally and sown in beds from the middle of June, 1930. Each sample of seeds was sown separately. Early in July, when the plants were sufficiently high, they were transplanted into larger beds in the insectary garden and also in the flower beds surrounding the building. The different horticultural varieties were kept separate and labelled accordingly.

A wire-gauze cage, $20\frac{1}{4}$ ft. $\times 10\frac{1}{4}$ ft. $\times 8\frac{1}{4}$ ft., of 20 to the inch mesh, was divided into two halves by a barrier in the soil of treated planking. In one of these halves the soil was dug over a depth of about $2\frac{1}{2}$ feet, weeded, mixed with garden manure, baked over a fire, and then sifted. This procedure was followed by fumigation with cyanogas dust. The soil of the second half was also turned over, but was left unsterilised.

The two halves were divided into rectangular strips, in each of which various samples of Zinnia seeds used in the insectary garden were sown on the 18th June, 1930. The seeds were allowed to germinate *in situ*, germination occurring in all the plots within three days. By the middle of July the seedlings in both halves of the cage began to show the symptoms of leaf-crinkle. These plants contracted the disease in a more severe form than those grown in the open. The small mesh of the gauze eliminated all insect life except minute Aleurodidae (*Bemisia gossypiperda*), which infested the caged Zinnias in abundance.

The obvious conclusion from this preliminary experiment was that these Aleurodids were involved in the spread of the disease. To test this inference transmission experiments were initiated in early August, 1930, and continued in 1931. Zinnia seeds were sown in 9-inch sterilised pots containing sterilised soil, and were germinated under glass bell jars or chimneys the bases of which were firmly implanted in the soil and the tops covered with securely fine muslin, thus forming an insect-proof chamber. These pots were kept inside a wire-gauze cage,

similar to that used for the preliminary experiment. All the seedlings grew well and were thinned out, only one seedling per pot being retained. Later on the condensation of moisture upon the sides of the bell jars and chimneys, combined with deficient ventilation, apparently caused the plants to become sickly. The preliminary experiments conducted with these plants are discussed below (III, *b*).

These difficulties led to the trial of simply designed cylindrical cages consisting of a detachable tin cylinder at the bottom, tin lid, and a supporting framework surrounded by muslin. A series of experiments were conducted in these cages (III, *c*). They also proved, however, to be unsatisfactory, owing to their small size and to the heating of the lids by the sun and the obstruction of light. In 1931, the work was therefore continued with further modifications in technique.

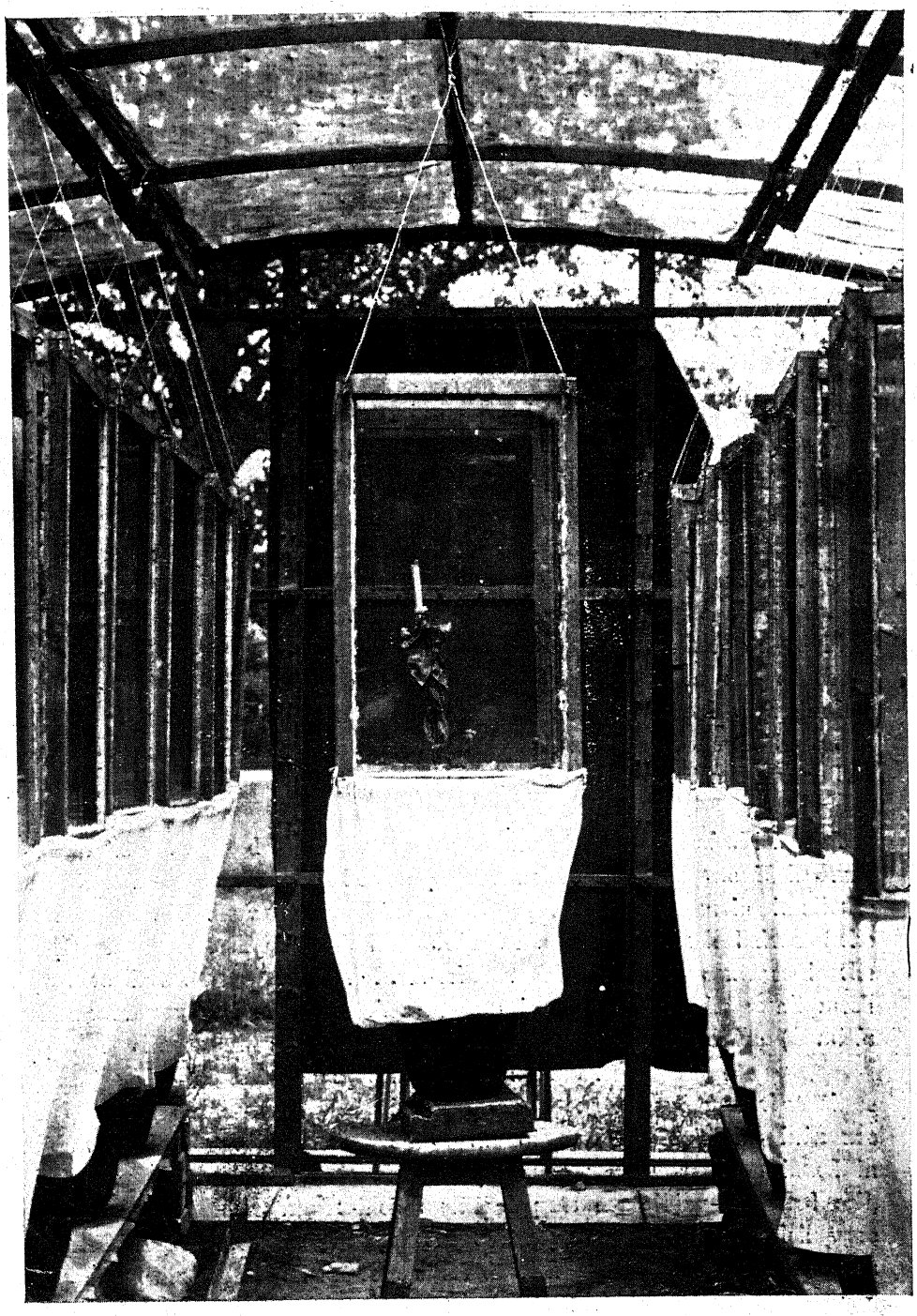
Thirty new cages were made. It will be seen that each unit of the equipment (Plate X) included (1) a wooden frame fitted with glass panes and open at the lower end, (2) a muslin bag open at both ends, and (3) a 10-inch pot having a tin cylinder pushed inside the soil of the pot and enclosing the plant. Each frame was suspended by wires within a large outdoor wire-gauze cage. One end of the bag was secured to the open end of the frame by means of a tape threaded through the hem of the bag; the other and narrower end was similarly secured to a surrounding projection a little below the top of the cylinder, thus making it possible to water the plant without exposing it to accidental infections. This equipment ensured the continuation of the work under adequately controlled and better conditions (III, *d*).

The Aleurodids used in the experiments (described in III, *b-d*) were collected from diseased Zinnias growing in a large cage in the insectary and then transferred to healthy plants grown from seeds under controlled conditions. In the controls no white-flies were used. The more refined technique of using white-flies bred under controlled conditions and of employing uninfected white-flies in the control experiments was not possible, but such methods should be employed in future work.

(*b*). *Results of preliminary experiments in 1930.*

The data regarding the preliminary experiments referred to above (III, *a*) are as follows:—

Plant A—4th-7th August	. . .	98 white-flies released.
11th August	. . .	Upper leaves showed signs of curling.
12th ,,	. . .	20 more white-flies released.
15th ,,	. . .	Leaves completely crinkled.
2nd September	. . .	Plant died.
Plant E—12th-27th August	. . .	367 white-flies released.
25th August	. . .	Slight curling of leaves noticed.
2nd September	. . .	Leaves thoroughly crinkled.
10th ,,	. . .	Plant died.



Cages used for transmission experiments and a plant of *Zinnia elegans* with severe symptoms of leaf-curl. Note the extremely dwarfed flowers.

None of the Aleurodid-free control plants (B, C, D), grown under similar conditions, contracted the disease.

(c) *Results of further experiments in 1930.*

The results of the experiments (four were later eliminated from the series) conducted in simple frame cages are given in Table I. It will be seen that the white-flies produced mild symptoms of leaf-curl in 4-16 days and severe symptoms in 16-31 days, while the Aleurodid-free control plants grown under similar protected conditions, remained free of crinkle with normal leaves and flowers. Plant No. 5 (*control*) emphasizes this fact, in which 24 white-flies were accidentally released on 17th September.

TABLE I.

Results of inoculations to Zinnia elegans *with viruliferous white-flies in 1930.*

Serial number	Date of inoculation	No. of white-flies	Period when flies released (days)	Date observed	
				First symptoms	Death of plants
	September 1930			1930	
1	10th . .	240	23	22nd September	13th October 1930
Control	10th . .	—	—	*Nil*	*Nil*
2	12th . .	265	22	22nd September	3rd November 1930
Control	12th . .	—	—	*Nil*	*Nil*
5	15th . .	174	16	22nd September	3rd November 1930
Control*	15th . .	—	—	3rd October	9th December 1930
6	15th . .	51	10	1st October	20th November 1930
Control	15th . .	—	—	*Nil*	*Nil*
7	15th . .	150	6	8th October	9th December 1930
Control	15th . .	—	—	*Nil*	*Nil*
9	15th . .	256	9	18th October	21st January 1931
Control	15th . .	—	—	*Nil*	*Nil*

* Control to plant No. 5 became diseased on account of accidental infection.

(d) Results of experiments in 1931.

The results of transmission experiments conducted in 1931 are discussed in Table II. The results appear to prove that *Bemisia gossypiperda* is undoubtedly the vector of the leaf-curl of Zinnia. In the experiments proper the plants contracted the disease within 6-25 days; in the controls they remained healthy for a long period. The accident in the case of Plant No. 7 (control) due to the entrance of the white-flies produced results in general conformity with those of the other experiments. The symptoms were not marked, but this may be due to the fact that the Aleurodids found in the cage were not previously collected on diseased Zinnias as in the case of those used for the experiments proper. Experiment 3 indicates that a single white-fly can transmit the disease to a healthy plant with results as fatal as when many are used. This is in accordance with the observations of Kirkpatrick [1931] who states that if "transmission of the disease is obtained with a single white-fly, the resulting symptoms of crinkle are liable to be just as severe as if a large number is used to transmit the infection." Similar results have been noted in the case of various other vectors of viruses [Smith, 1931]. Pruning (with a sterilised knife) appeared to have no effect on disease transmission.

TABLE II.

Results of inoculations to Zinnia elegans *with viruliferous white-flies in 1931.*

Serial number	Date of inoculation	No. of white-flies	Period when flies released (days)	Date observed	
				First symptoms	Death of plants
	1931			1931	1931
1	19th May . .	150	5	30th May . .	25th June
2	21st May . .	106	2	Do. . .	2nd July
3	4th June . .	1	1	13th July . .	21st September
4	Ditto . .	265	8	6th July . .	31st July
Control	Ditto . .	..	..	*Nil*	*Nil*
5	Ditto . .	280	5	6th July . .	31st August
Control	Ditto . .	..	..	*Nil*	*Nil*
6	Ditto . .	150	6	13th July . .	22nd August
Control	Ditto . .	..	..	*Nil*	*Nil*
7	Ditto . .	260	3	20th July . .	31st August
Control	Ditto . .	..	..	..	..
9	Ditto . .	250	3	22nd July . .	9th September
Control	Ditto . .	..	..	*Nil*	*Nil*
10	Ditto . .	363	4	27th July . .	21st September
Control	Ditto . .	..	..	*Nil*	*Nil*

NOTE.—No controls were used for Plants Nos. 1 to 3.

(*e*) *Results of experiments with newly-emerged Aleurodids reared from nymphs fed on diseased plants.*

In some experiments adult Aleurodids were bred from nymphs fed on diseased Zinnia plants and released, immediately after emergence, on healthy plants. The results are given in Table III, and suggest that adults of the Aleurodid concerned require to feed on a diseased plant before they become infective. Kirkpatrick's experiments [1931], however, indicate the opposite conclusion that white-flies which have become viruliferous in their larval stages, but have not fed as adults on diseased plants, are efficient vectors of leaf-curl. Our experiments are insufficient to enable this conclusion to be contradicted in so far as the relation between Aleurodids and leaf-curl of Zinnia is concerned.

The diseased plants bearing puparia and nymphs were kept inside a glass cage enclosed in a dark cover, which was manipulated to allow the entry of a small shaft of light. This attracted the newly-emerged white-flies to the illuminated portion of the cage, where they were collected for transfer. The risk of feeding before transfer was not eliminated, but nevertheless none of the healthy plants to which the adults were transferred gave indications of the disease. The general technique was as in the other experiments.

TABLE III.

Results of inoculations in Zinnia elegans *with adult Aleurodids bred from nymphs fed on diseased Zinnia plants.*

Serial number	Date of inoculation	No. of adults	Period when flies released (days)	Symptoms
	1931			
A . . .	15th July . . .	120	3	Healthy
B . . .	Do. . . .	87	4	Healthy
C . . .	Do. . . .	163	7	Healthy
D . . .	Do. . . .	150	5	Healthy
E . . .	Do. . . .	47	4	Healthy
17 . . .	4th September . .	80	5	Healthy
19 . . .	5th October . .	483	6	Healthy
20 . . .	Do. . . .	237	13	Healthy

IV. Summary.

1. Leaf-curl of garden Zinnias is characterised by the thickening of the lower surface of the veins, preceded by curling of the leaf-blades. Cessation of growth and eventual death follows infection.

2. The disease closely resembles the leaf-curl of cotton as studied by Kirkpatrick in the Sudan.

3. Aleurodidae (*Bemisia gossypiperda*) white-flies were vectors of the disease.

4. After the transmission experiments were concluded it was discovered that the vectors of leaf-curl of Zinnia and cotton belong to the same species (*Bemisia gossypiperda*).

5. Freshly-emerged adult white-flies did not transmit the disease, though fed in the nymphal stages on diseased Zinnias. This result requires further investigation.

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DEVELOPMENT AND SHEDDING OF LEAVES OF COTTON.

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INTRODUCTORY.

The literature on the development, growth and shedding of leaves of cotton is scanty. So far as the writer is aware, experimental work on the development of leaves has only been done by Zaitzev [1925-27]. A translation of his paper entitled " The effect of Temperature on the development of the cotton plant " has been published by the Empire Cotton Growing Corporation. In this paper Zaitzev has shown that in the very early stages of the growth of the seedling the intervals between successive pairs of leaves were alternately long and short. " The periodic difference in the intervals between successive pairs decreases both in the course of growth and also with later dates of sowings". He has also shown that the unfolding of successive leaves on the main stem is mainly dependent on temperature. At higher temperatures fresh leaves unfold more quickly.

The importance of leaves for the proper development of the plants is patent. The experiments of Ludwig [1927] showed that artificial defoliation done early in the season resulted in reduced yields and weak lint. Haller and Magness [1925] found in apples that " Other factors being constant, the growth of apples is directly correlated with the leaf area upon which the apples are able to draw for carbohydrates."

It would thus be of interest to study the growth and shedding of leaves of the cotton plant. Such studies are likely to bring forth results of great interest as the occasional 'failures' of the cotton crop in the Punjab, whatever their causes, are ultimately manifestations of malnutrition. It is significant that 'failures' are always preceded by premature yellowing, reddening and shedding of leaves.

DEVELOPMENT OF LEAVES.

The first foliage organs of the young seedlings are the cotyledons. The cotyledons persist on the plant for various lengths of time and the differences in the ages of cotyledons of different varieties are statistically significant as shown in Table I. Full data are given in the appendix.

TABLE I.

Analysis of variance.

Variance due to	Degrees of freedom	Sum of squares	Mean square
Varieties	3	583·90	194·63
Error	196	3390·40	17·30
Total .	199	3974·30	... z=1·2100

The one per cent. value of z is only 0·6651 showing thereby that the effect of varieties is clearly significant [Fisher, 1930].

The mean ages (in days) of cotyledons were as follows :—

Early Strain	4 F	289 F	Mollisoni	S. E.
37·89	41·39	42·41	39·93	±0·59

It is of interest to note that the cotyledons of *G. stocksii* and *G. kirkii* grown at Lyallpur persisted on the plants for more than 2 and 5 months respectively.

The cotyledons do not come out from a single node on the young axis, but one comes out slightly above the other. Both the cotyledons are rarely shed on the same day. There is, in fact, a difference of 3 to 10 days in their ages. The sequence of shedding, however, varies and the chances of the upper or the lower cotyledons falling off first in different plants are practically equal as illustrated by the following data collected on another occasion.

Average age of the upper cotyledon of 4 F = 51·65 days.

Average age of the lower cotyledon of 4 F = 51·25 days.

The cotyledons are followed by true leaves. In order to find out the interval between the appearance of successive leaves, twenty plants each of 4 F., 289 F, Early Strain and Mollisoni were kept under constant observation and every young leaf which separated from the growing point of the main stem was properly labelled and recorded. The average interval between the appearance of successive leaves is shown graphically in Figure 1. From the different curves in this figure, it is seen that there is some periodicity in the intervals but the long and short intervals do not alternate very regularly.

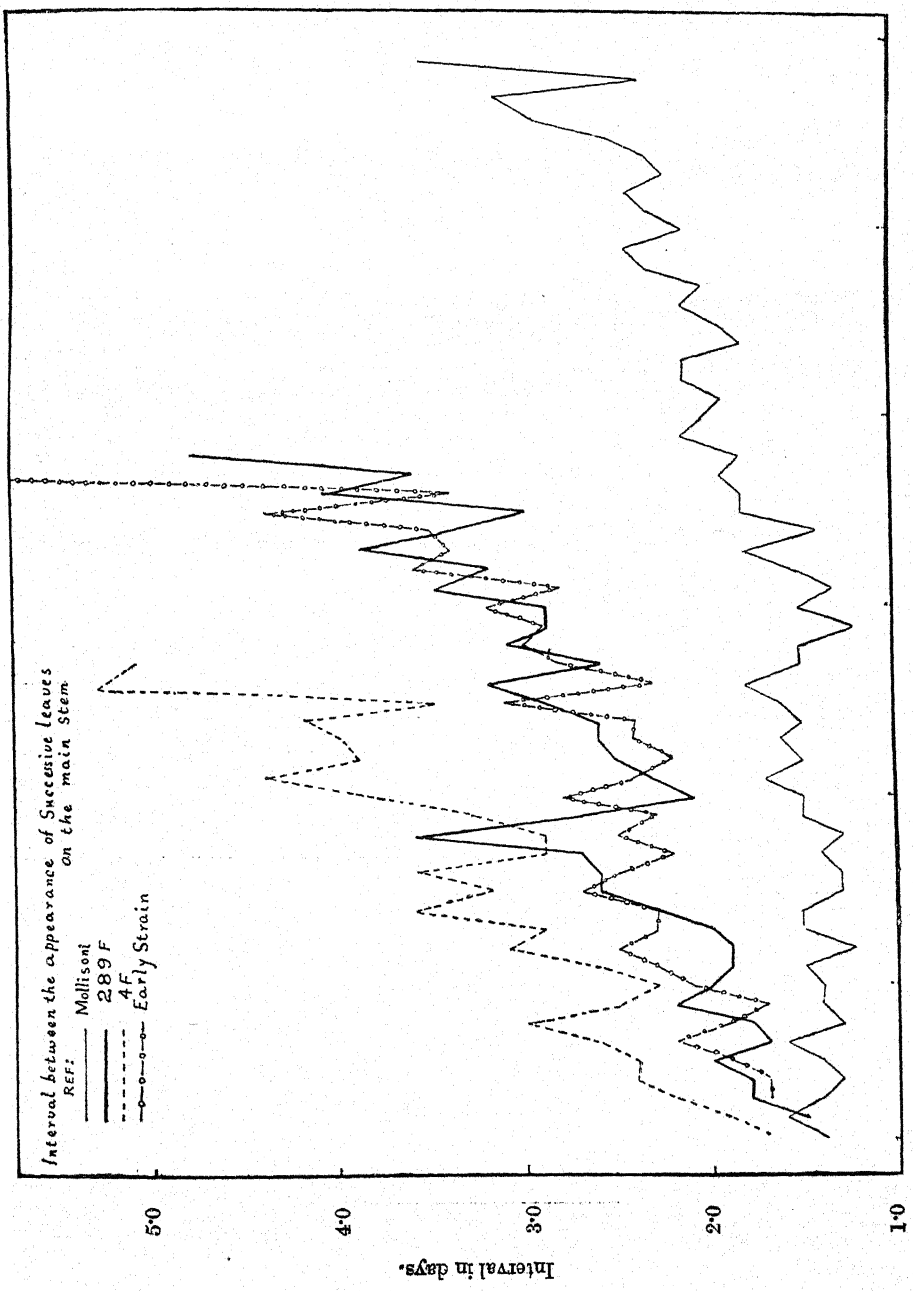
Interval between the appearance of Successive leaves on the main Stem
REF:
Mollisoni
289 F
4F
Early Strain
Interval in days.
5·0
4·0
3·0
2·0
1·0
15
23
33
43
53
63
73
Number of the nodes on the main stem.

Fig. 1.

There has been found a fairly high correlation between the rapidity of appearance of the successive leaves on the main stem and the atmospheric temperature. At higher temperature the leaves appeared at short intervals and as the temperature went down these intervals become comparatively longer. The coefficient of correlation was $+0{\cdot}85 \pm 0{\cdot}045$.

Zaitzev also found a correlation between the succession of leaves on the main stem and the length of the internodes. In his case there was a very high correlation between these two factors, especially in the young stages.

The internodes of all the plants under observation were measured, and it was found that the internode between the cotyledons and the first leaf was in every case very long. This long internode was always followed by a short one. After the second internode the length remained fairly constant till about the 15th internode. After this stage the length again increased and reached a maximum in the middle of the plant, after which the length again decreased, and the last internode was in all cases less than one cm. In the present case, however, the correlation coefficient between the length of the internodes and the interval between the appearance of successive leaves was found to be very small and statistically insignificant ($r = +0{\cdot}09 \pm 0{\cdot}002$).

Trought [1931] has already shown that "the increase in length of the monopodia conforms very nearly to the increase in length of the main stem", and therefore the increase in height of the main stem "can be taken as an index of the general elongation of the plant". It may therefore be inferred that the general sequence of the appearance and the interval between the successive leaves on the monopodia may also be similar to that of main stem. Therefore no observations were taken on the appearance of leaves on the branches of the plants, but the total number of leaves on 5 average plants was counted once a week and these figures are given in Table II.

TABLE II.

Average number of leaves per plant.

Week ending	No. of leaves			
	4 F	289 F	Early Strain	Mollisoni
7th July	214	164	116	171
14th ,,	242	193	147	186
21st ,,	280	239	181	235
28th ,,	304	263	174	303
4th August	321	339	211	372
11th ,,	301	314	232	396
18th ,,	329	353	222	463
25th ,,	331	278	238	469
1st September	263	277	203	507
8th ,,	270	285	223	560
15th ,,	270	282	212	604
22nd ,,	223	233	204	575
29th ,,	211	218	196	538
6th October	202	197	159	528
13th ,,	182	181	172	497
20th ,,	151	171	150	392
27th ,,	141	160	142	322
3rd November	121	146	134	288
10th ,,	82	116	103	239
17th ,,	78	113	96	232
24th ,,	50	91	80	165
1st December	69	100	89	224
8th ,,	74	88	70	245
15th ,,	60	79	77	257

The figures given in Table II are interesting. There is a marked contrast between the Desi and the American varieties. While in the case of all American cottons the maximum number of leaves were present during August, in the case of Mollisoni, the maximum number of leaves were present during September. Moreover, the Mollisoni plants always had a much bigger number of leaves than the plants of 4 F, 289 F and Early Strain. Between the three latter cottons, there appears to be some advantage in favour of 4 F, while there was not much difference between the other two cottons. Another point worth mentioning is that in the case of American cottons the number of leaves steadily decreased from September onwards, but in Mollisoni, the number at first decreased but again started to increase from the beginning of December. This increase in number was due to the fact that Mollisoni plants started to sprout again from the beginning of December, while the plants of American cottons did not sprout so soon.

Growth of leaves.

The next point to be considered is the daily rate of growth of leaves. The writer has not been able to find any reference on this subject in cotton or other plants, but the rate of growth in leaves represents the daily addition to the photosynthetic area and is therefore important.

The rate of growth of leaves has been investigated in the case of all the four varieties, *viz.*, 4 F, 289 F, Early Strain and Mollisoni. For this purpose twenty primary young leaves just separating from the growing point were selected on twenty different plants and the length of each lobe and the petiole as well as the greatest breadth of each lobe was measured daily. Since this work involves laborious measurements, only two varieties were kept under observation in one year. The leaves of 4 F and Mollisoni were measured in 1929 and those of 289 F and Early Strain in 1930.

It has not been possible to measure the rate of growth of all the leaves, and the observations here recorded were made on leaves produced during the period of most vigorous growth.

In order to be able to tabulate systematically the growth in length and breadth, the different lobes of the leaves, ventral surface of the leaf upwards, were numbered serially as shown in the following diagram.

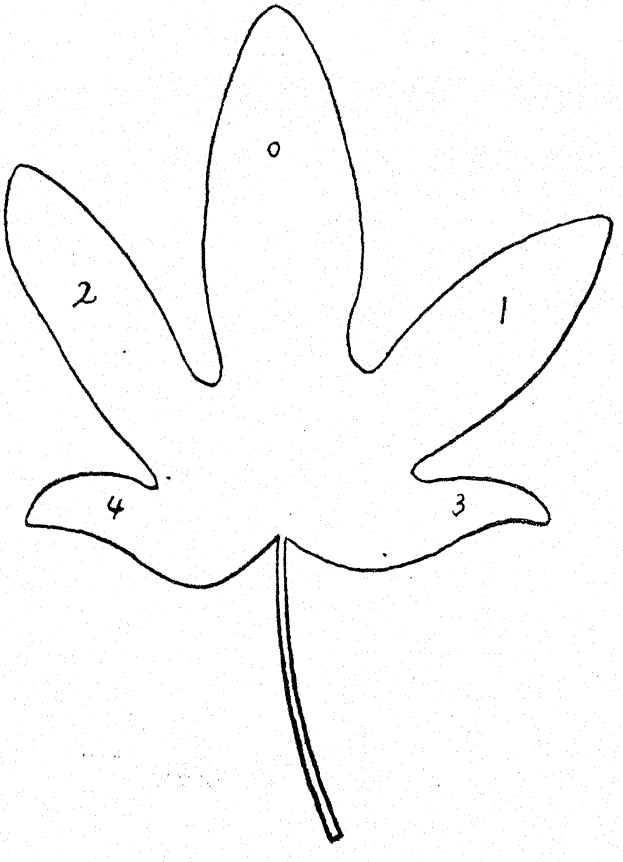

Fig. 2.—Diagrammatic representation of cotton leaf showing the order in which the lobes were measured.

Curves of the average rate of growth are shown in Figures 3, 4, 5 and 6. In considering these figures it must be borne in mind that all the varieties have not been measured in one year.

It is seen from Figures 3 and 4 that 4 F leaves took full one month to reach full size while those of Mollisoni were full grown after 20 days.

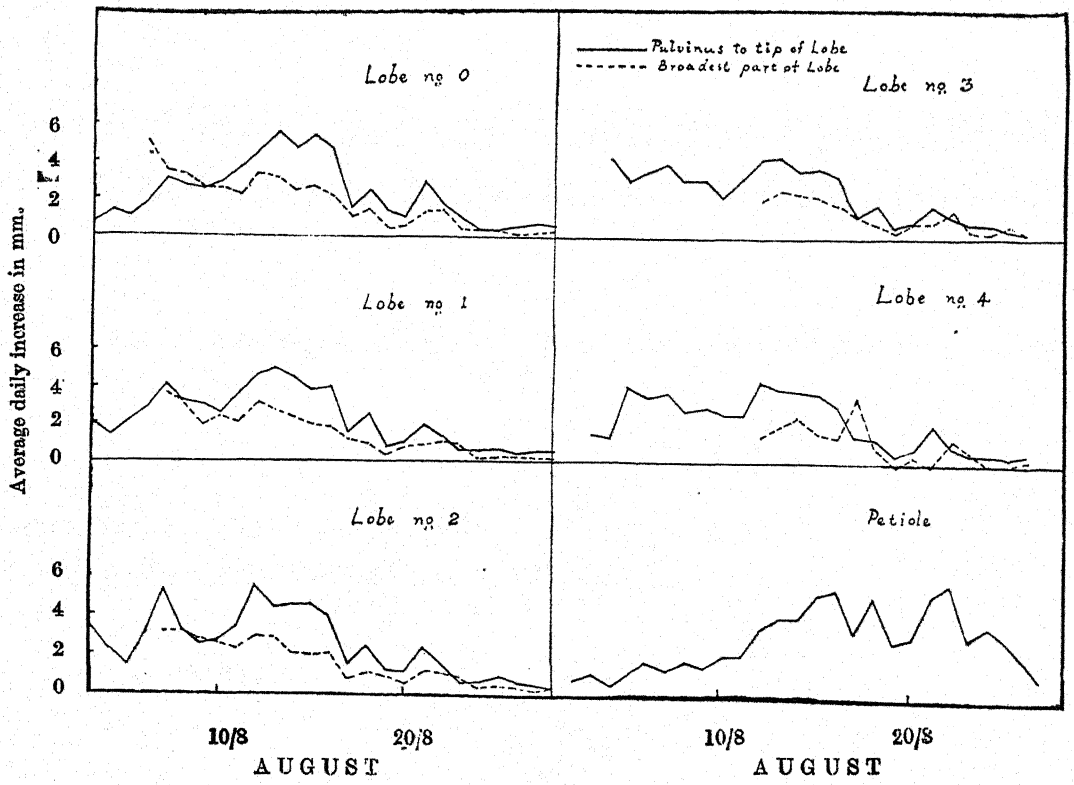

Fig. 3.—Leaf measurements of 4 F (1929).

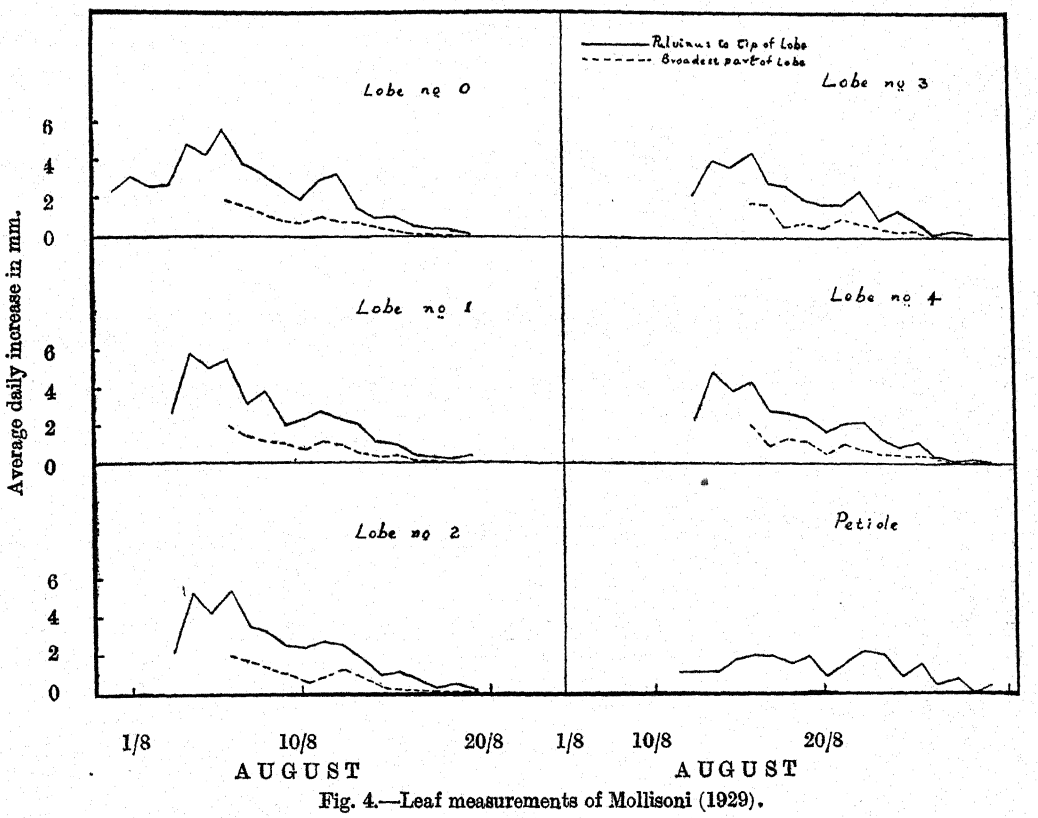

Fig. 4.—Leaf measurements of Mollisoni (1929).

Another point of interest is that the petiole took exactly the same time to attain full size as the lamina and that there was a high correlation between the length of the petiole and the length of the midrib ($r = +0{\cdot}895 \pm 0{\cdot}034$). A similar correlation between length of midrib and the length of petiole has already been reported by the writer in a hybrid population [Afzal, 1931].

The average rate of growth of leaves of 289 F and Early Strain (measured during 1930) is shown in Figures 5 and 6. The leaves of these cottons had a higher

rate of growth than 4 F, and took 21 days to attain full size. Here again the petiole attained full size along with the lamina.

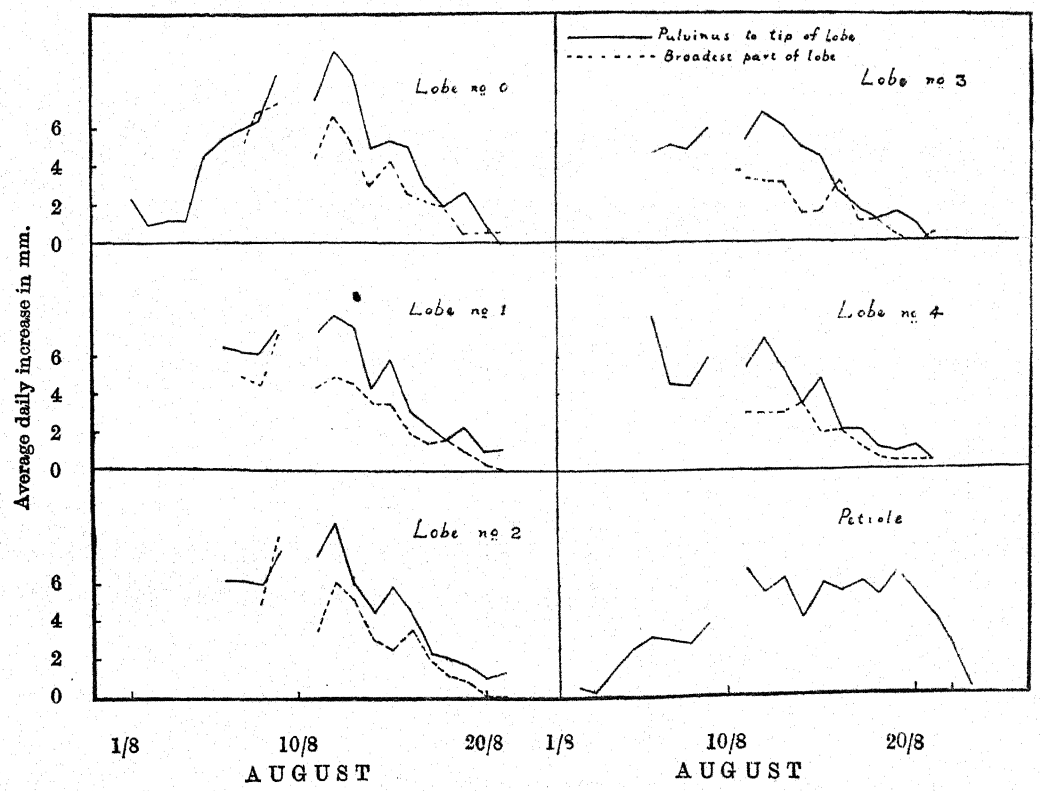

Fig. 5.—Leaf measurements of 289F (1930).

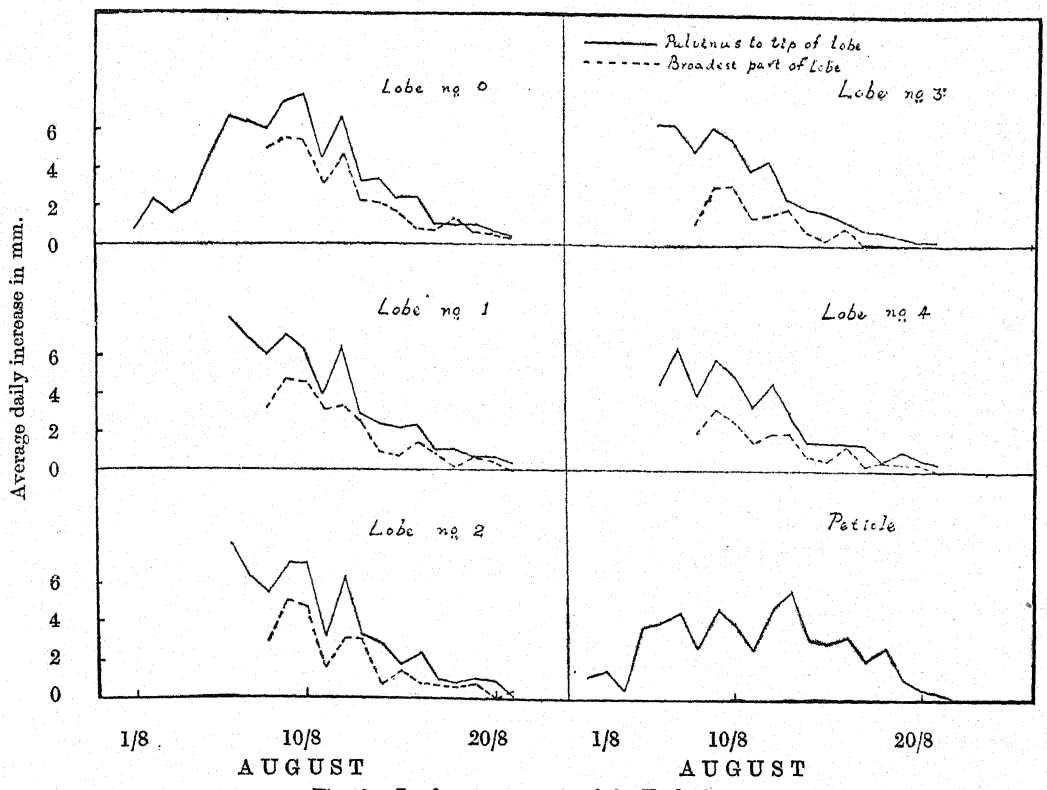

Fig. 6.—Leaf measurements of the Early Strain (1930).

The daily fluctuations in the rate of growth will now be considered. It is at once clear from the different figures, that there is a remarkable resemblance of the different curves of each year in this respect. Whatever factor retarded or accelerated growth in any one lobe of the leaf, also had a similar effect on the different lobes of the same leaf and on the leaves of other varieties. It is, however, too early to state the causes of the daily fluctuations in the growth of leaves, but it is hoped that the experiments now in progress at the Cotton Research Laboratory will throw light on this phenomenon.

Shedding of leaves.

The phenomenon of leaf shedding in cotton has, so far as the writer is aware, not received much attention. A good deal of work has, however, been done on this problem in other plants, especially the deciduous trees.

Schimper [1903] mentions certain cases where the leaf fall could be directly traced to external causes, such as, low winter temperatures in temperate regions or dry seasons in the tropics. On the other hand he found certain trees in which the leaf fall was not due to external agencies, but was brought about by internal causes. He also observed a relationship between soil moisture and leaf fall, and states that a greater amount of moisture in the soil delays leaf fall. Veihmeyer and Hendrickson [1927] however, state that young peach and prune trees on moist soil held in containers dropped their leaves at the same time in fall as those on drier soil. They further state that defoliation could be brought about during periods of high evaporation by withholding water until the trees wilted. Inamdar and Shrivastava [1927] have traced a correlation between leaf fall and the increased capacity of the wood for conducting water. They came to the conclusion that increased demand for transpiration is met with by increased capacity of wood for conducting water. Whether this is true in the case of cotton plant or not is a subject of investigations still under way. Thomas [1922] on the other hand has pointed out that citrus trees on " heavy soil may become stunted where excessively irrigated. The leaves turned more or less yellow and many of these fell prematurely."

No attempt has so far been made to find out the causes of leaf shedding in cotton at Lyallpur, but the observations herein recorded only show the ages at shedding of the different leaves on the main steam.

It has been found in cotton that the age of primary leaves at shedding varied with the season (Table III).

TABLE III.

Variation of age of leaves at shedding with season.

Year	Date of appearance of leaf	Average age at shedding in days			
		Mollisoni	4 F	289 F	Early Strain
1929	30th July . .	64·9	67·15	..	..
1930	25th July . . .	77·35	76·33	78·0	85·5
	8th August . .	115·8	106·80	117·2	105·33
	22nd August . .	93·15	105·7	101·95	88·7
	5th September . .	..	78·2	88·90	..

The figures obtained during 1930 showed a very wide variation in the age of leaves at shedding during the season. Therefore in order to obtain more data, the age of primary leaves throughout the 1931 season was recorded. The figures (Table IV) are averages of twenty plants of each variety.

TABLE IV.

Average age (days) of primary leaves in different varieties (1931).

Node No.	Mollisoni	Early Strain	289 F	4 F
15	39·08	39·40	36·69	45·76
16	40·25	45·27	42·63	47·27
17	43·50	46·31	45·10	47·80
18	44·85	42·30	50·25	50·01
19	45·40	45·00	51·05	47·20
20	46·84	46·75	48·50	49·42
21	46·05	53·55	51·05	46·20
22	48·66	50·60	49·20	44·75
23	47·85	47·15	43·43	47·50
24	49·61	49·68	42·85	54·00
25	50·05	43·45	41·95	59·06
26	46·94	40·70	40·80	59·12
27	48·84	40·05	37·90	65·05
28	49·99	37·76	41·35	71·15
29	51·34	37·10	40·40	68·80
30	53·15	42·95	42·22	74·10
31	53·17	43·74	42·68	80·30
32	55·17	49·27	49·05	89·53
33	55·16	54·18	52·95	81·85
34	54·39	55·43	61·33	90·05
35	61·84	61·14	60·82	93·80
36	60·28	65·25	51·39	94·37

Node No.	Mollisoni	Early Strain	289 F	4 F
37	63·19	60·95	67·00	93·89
38	59·95	69·95	60·88	101·40
39	61·30	72·41	73·29	86·07
40	62·47	70·00	76·14	85·75
41	61·72	76·29	83·17	81·50
42	61·72	85·68	86·95	79·75
43	61·45	88·79	88·79	..
44	61·40	83·00	93·57	..
45	57·00	97·89	99·42	..
46	60·61	101·26	102·16	..
47	67·47	93·42	98·06	..
48	67·74	99·33	102·25	..
49	71·83	87·29	102·00	..
50	72·53	82·50	103·77	..
51	72·22	82·66	101·60	..
52	77·00	..	106·29	..
53	70·47	..	99·60	..
54	77·30	..	90·33	..
55	82·50	..	..	..
56	79·53	..	..	..
57	87·79	..	..	..
58	87·19	..	..	..
59	92·77	..	..	..
60	98·00	..	..	..
61	93·91	..	..	..
62	96·48	..	..	..
63	106·05	..	..	..
64	104·22	..	..	..
65	95·5	..	..	..

The figures in Table IV confirm the data obtained during 1930 (Table III). The leaves at lower internodes were comparatively short lived. At higher nodes the age of leaves increased till a maximum was reached near the top of the plant. The very topmost leaves again had a comparatively short duration of life.

The number of leaves shed per plant have also been worked out and the figures (Table V) are average number of primary leaves shed per plant per week. For this purpose the same twenty plants per variety were used.

TABLE V.

Average number of primary leaves shed per plant per week.

Week ending	Mollisoni	4 F	Early Strain	289 F
16th July	0	0	0	0·2
23rd „	0·7	0·7	1·4	1·0
30th „	3·7	0·8	1·8	1·0
6th August	3·1	2·9	4·2	3·5
13th „	3·4	3·9	2·7	4·5
20th „	1·9	0·9	2·7	3·4
27th „	3·4	1·3	2·2	2·3
3rd September	2·7	1·3	1·3	0·9
10th „	1·1	0·3	1·0	0·8
17th „	3·1	1·6	0·9	1·5
24th „	6·9	1·5	1·5	1·9
1st October	1·5	1·3	1·9	1·3
8th „	0·2	0·1	1·3	0·4
15th „	3·1	1·5	1·8	1·5
22nd „	3·0	1·6	1·3	0·7
29th „	2·4	1·3	0·8	1·1
5th November	0·9	1·5	0·8	0·6
12th „	1·2	0·9	0·6	0·9
19th „	1·6	0·8	1·0	1·2
26th „	0·8	0·2	0·6	0·9
3rd December	0·9	0·4	0·5	0·5
10th „	0·7	0·3	0·6	0·8

Week ending	Mollisoni	4 F	Early Strain	289 F
17th December	1·8	0·5	1·0	1·1
24th „	1·6	0·5	0·2	0·3
31st „	0·2	0·1	0·1	0·7
7th January	0·5	0·3	0·4	1·4
14th „	0·8	0·3	0·3	0·7
21st „	1·1	..	..	0·2
28th „	1·0	..	..	0·4
4th February	0·5	..	..	0·2
11th „	..	..	..	..
18th „	..	..	..	..

Bailey [1930] has stated that in the Sudan most of the shedding of leaves of cotton occurs in December and January (*i.e.*, 4 or 5 months after sowing). At Lyallpur, on the contrary, more leaves were shed during August and September (*i.e.*, 3rd and 4th month after sowing). It may also be observed that the shedding of leaves continued at a high rate much later in the Mollisoni cotton as compared to the three American varieties.

Summary.

The development and shedding of leaves of four varieties has been studied. The ages of the cotyledons were significantly different in the various varieties.

The appearance of the successive leaves on the main stem was affected by the prevailing temperature, but there was no correlation between the intervals in the appearance of successive leaves on the main stem and the length of the internodes.

The age of leaves at the time of shedding varied considerably during the course of the season.

Acknowledgments.

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Appendix.

The ages of the cotyledons referred to in Table I were reckoned from the day of the emergence of the seedlings above ground to the day of their shedding. Fifty plants were selected in each variety and the ages of both the cotyledons along with the means are given in Table VI.

TABLE VI.

Age of cotyledons in days.

Plant No.	Early Strain			4 F			289 F			Mollisoni		
	Cotyledon		Mean	Cotyledon		Mean	Cotyledon		Mean	Cotyledon		Mean
	1	2		1	2		1	2		1	2	
1	34	35	34·5	40	41	40·5	41	52	46·5	49	50	49·5
2	34	34	34·0	36	41	38·5	38	40	39·0	44	46	45·0
3	36	36	36·0	36	44	40·0	36	38	37·0	35	45	40·0
4	37	45	41·0	37	50	43·5	35	39	37·0	35	36	35·5
5	36	36	36·0	36	43	39·5	33	35	34·0	30	48	39·0
6	34	36	35·0	37	47	42·0	38	38	38·0	31	45	38·0
7	35	45	40·0	34	41	37·5	38	42	40·0	30	35	32·5
8	33	37	35·0	45	45	45·0	36	36	36·0	41	42	41·5
9	36	37	36·5	46	46	46·0	34	36	35·0	43	46	44·5
10	35	37	36·0	37	39	38·0	33	36	34·5	43	44	43·5
11	39	39	39·0	36	38	37·0	40	41	40·5	30	42	36·0
12	38	38	38·0	36	36	36·0	34	36	35·0	44	45	44·5
13	39	40	39·5	49	49	49·0	34	36	35·0	43	45	44·0
14	36	39	37·5	38	42	40·0	33	36	34·5	38	42	40·0
15	38	41	39·5	37	41	39·0	47	47	47·0	34	43	38·5
16	39	43	41·0	37	39	38·0	37	37	37·0	37	44	40·5
17	38	43	40·5	38	43	40·5	36	41	38·5	44	50	47·0
18	35	39	37·0	42	43	42·5	39	46	42·5	42	43	42·5
19	42	42	42·0	47	47	47·0	53	53	53·0	36	36	36·0
20	33	35	34·0	37	42	39·5	35	41	38·0	38	41	39·5
21	34	37	35·5	36	46	41·0	36	38	37·0	33	35	34·0
22	38	40	39·0	36	43	39·5	39	43	41·0	33	41	37·0
23	36	37	36·5	38	42	40·0	36	44	40·0	30	39	34·5
24	36	39	37·5	38	38	38·0	34	41	37·5	30	38	34·0
25	34	38	36·0	35	37	36·0	34	43	38·5	37	42	39·5

Plant No.	Early Strain			4 F			289 F			Mollisoni		
	Cotyledon		Mean	Cotyledon		Mean	Cotyledon		Mean	Cotyledon		Mean
	1	2		1	2		1	2		1	2	
26	36	37	36·5	37	50	43·5	40	47	43·5	30	46	38·0
27	33	45	39·0	45	47	46·0	37	37	37·0	30	32	31·0
28	37	38	37·5	43	46	44·5	45	45	45·0	30	41	35·5
29	34	39	36·5	44	46	45·0	41	44	42·5	43	43	43·0
30	39	39	39·0	45	45	45·0	39	39	39·0	46	48	47·0
31	34	38	36·0	53	53	53·0	45	45	45·0	37	46	41·5
32	36	36	36·0	36	36	36·0	37	44	40·5	43	44	43·5
33	39	39	39·0	41	45	43·0	38	47	42·5	44	46	45·0
34	38	41	39·5	35	44	39·5	53	53	53·0	43	46	44·5
35	36	39	37·5	36	46	41·0	43	45	44·0	46	47	46·5
36	38	40	39·0	46	46	46·0	40	49	44·5	34	44	39·0
37	38	40	39·0	39	45	42·0	38	47	42·5	33	44	38·5
38	35	41	38·0	44	44	44·0	52	55	53·5	43	43	43·0
39	38	40	39·0	39	45	42·0	44	52	48·0	32	33	31·5
40	40	48	44·0	42	48	45·0	52	52	52·0	33	35	34·0
41	35	39	37·0	34	47	40·5	38	45	41·5	42	46	44·0
42	43	43	43·0	41	45	43·0	44	48	46·0	44	46	45·0
43	36	36	36·0	38	41	39·5	46	46	46·0	41	42	41·5
44	33	45	39·0	44	47	45·5	37	43	40·0	40	42	41·0
45	41	41	41·0	47	47	47·0	45	45	45·0	33	33	33·0
46	36	41	38·5	48	48	48·0	38	38	38·0	40	41	40·5
47	36	40	38·0	41	48	44·5	43	43	43·0	43	44	43·5
48	35	36	35·5	45	47	46·0	39	45	42·0	43	46	44·5
49	36	36	36·0	54	54	54·0	43	45	44·0	31	32	31·5
50	38	39	38·5	47	47	47·0	36	39	37·5	32	43	37·5
Mean	..	..	37·89	..	..	41·39	..	..	42·41	..	..	39·93

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THE DEVELOPMENT OF FLOWER AND POLLEN IN JUTE.

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(With Plates XI and XII and one text-figure)

Investigations on the agronomy and improvement of the jute plant date from the beginning of this century, and many high yielding and superior strains of plants have been isolated by the Agricultural Department of Bengal. Finlow in a series of papers [1917, 1921, 1922], gives an account of the improvement of jute by pure line selection and also of the experiments relating to jute in Bengal. In a memoir published conjointly with Burkill [1912] he gives an account of the inheritance of red colour in *Corchorus capsularis* and also deals with the incidence of self-fertilisation in the plant. Practically all previous works refer to the improvement of the fibre or the plant, or to a report of its growth outside Bengal, [1921, 1926], and we look in vain for an account of an interspecific hybridisation or a cytological investigation. It has been mentioned by Maclean [1930] that "attempts to establish a cross between *Corchorus capsularis* and *Corchorus olitorius* have not so far met with success", but no evidence has been adduced nor investigations made to determine the factors responsible for the non-crossability of the plants. Difference in chromosome numbers of the parents as suggested by Denham [1924] in the case of cotton cannot possibly be the limiting factor here, as chromosome counts have shown that both species possess seven haploid chromosomes. Besides, in *Crepis* and in some other plants, interspecific crosses between plants having different chromosome numbers have been obtained.

The present investigation was undertaken in order to obtain some knowledge of the cytological behaviour of the plant which may be of use in hybridization, and in genetical investigations. Detailed study of the presynizetic and other stages of the microspore mother cells was not attempted as the material was found to be unsuitable for that purpose. A general outline of the development of the flower and pollen in *Corchorus olitorius* is presented.

Material and methods.

The material used in this investigation was obtained from plants grown in the University College gardens, from seeds obtained from the Director of Agriculture, Bengal. 'Chinsura Green', an olitorius variety was selected for study, as it is renowned for its fibre qualities, and is extensively grown in Bengal. The plants were grown during the summers of 1930 and 1931.

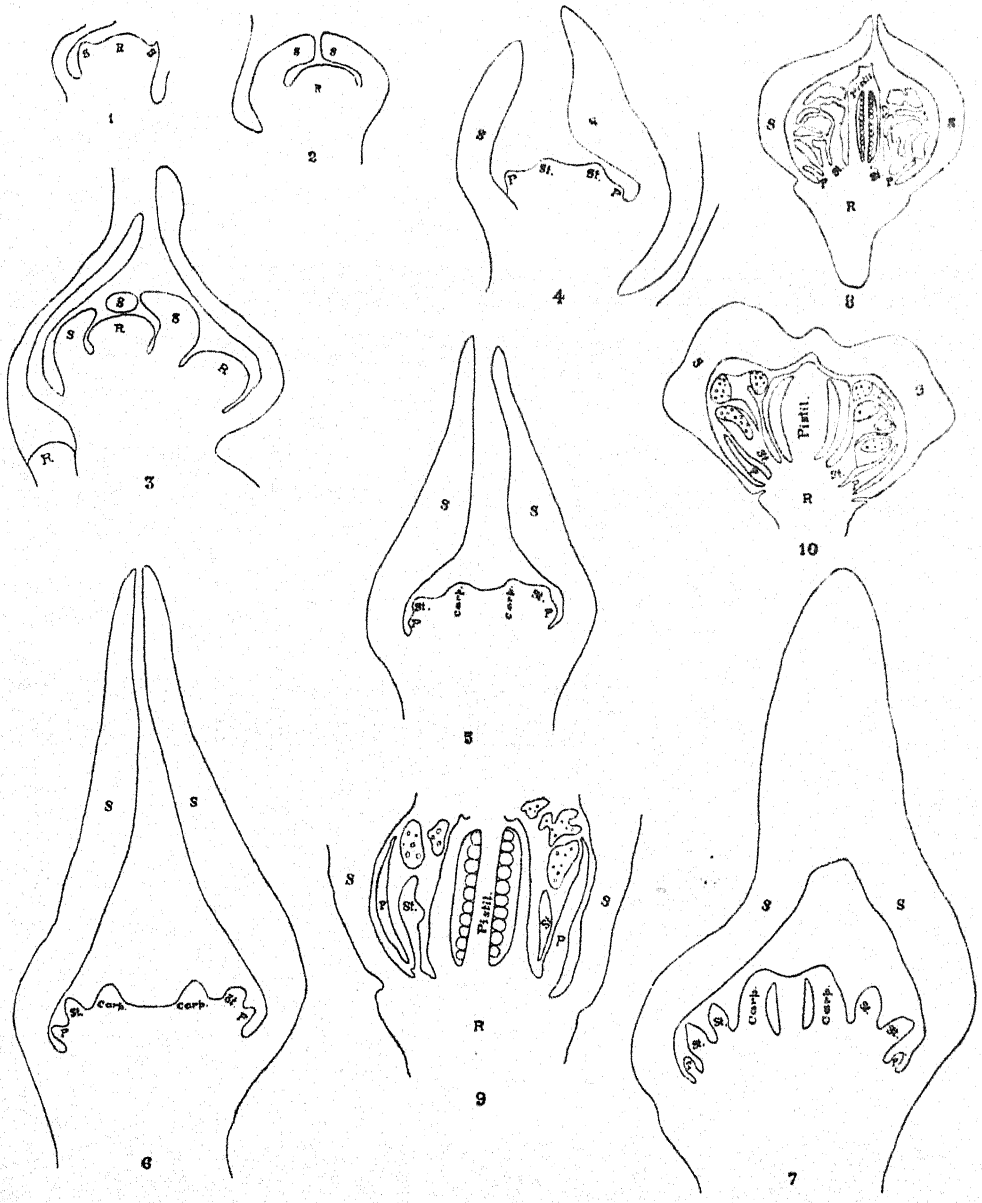
Preliminary examination of the anthers by Belling's [1923] aceto-carmine method gave an indication as to the size of the flower buds required for the study of meiosis. The fixing fluids used were Flemming's weak, Acetic-alcohol, and Allen's modified Bouin's fluid. Acetic-alcohol gave satisfactory results, but certain preparations showed contraction of the cytoplasm. Immersion in acetic-alcohol for a minute or so, followed by prolonged treatment in modified Bouin's solution, however, gave better results. Fixation was always done in the field on bright sunny days between 11 A.M. and 3 P.M. After dehydration and clearing the material was embedded in paraffin. Sections were cut 8, 10 or 12 μ thick, depending on the stage required for study. Haidenhain's iron-alum hæmatoxylin was used for staining. A number of preparations were counterstained with orange G.

The development of the flower.

The order of development of the floral parts appears to be centripetal, the sequence of development being sepals, petals, stamens and pistil. The first indication of the appearance of the flower bud appears to be a broadening of the growing point, which very soon assumes a convex and irregular outline. The sepals appear as five thick protuberances from the margin of the floral axis and develop rapidly. The petals and stamens appear a little later and almost simultaneously. In radial longitudinal, and in transverse sections, the individual primordium could be made out from their positions. Soon after and before the stamens have differentiated the carpel primordia are seen to arise from the central region of the flower. They are five in number and coalesce during their development to give rise to a pentalocular ovary.

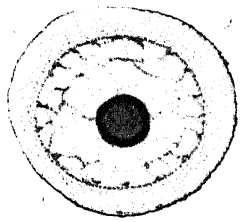
It is interesting to note that during the differentiation and development of the stamens and pistil, the sepals grow very rapidly and completely enclose the young flower, whereas, the growth of the petals becomes arrested soon after their appearance (Fig. 7). They resume growth when the other floral members have been differentiated. In fact, the renewed growth of the petals was noted when micros-

pore formation was nearly complete in the anthers. The figures below show the comparative development of the floral parts in *Corchorus olitorius*.



Stages in the development of the flower in jute. R. receptacles; S. sepal; P. petal; St. stamens; Carp. carpel; Figs. 1—9 are of Chinsura Green ; Figs. 1—7 × 190; Figs. 8 and 9 × 32; Fig. 10 is of *Corchorus capsularis* × 50. Note compressed nature of calyx. (Reduced to half.)

PLATE XI.



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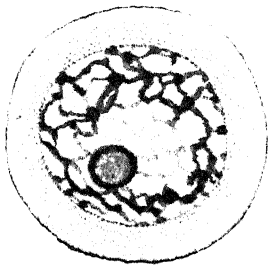

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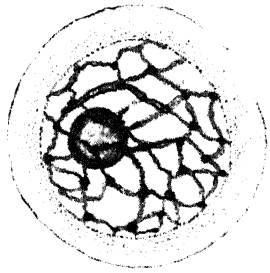

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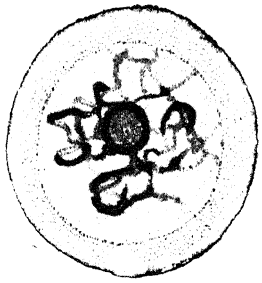

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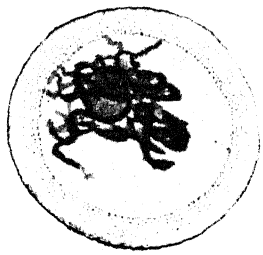

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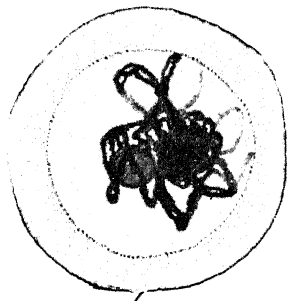

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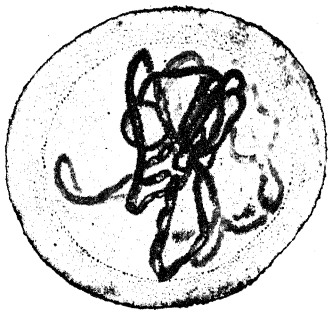

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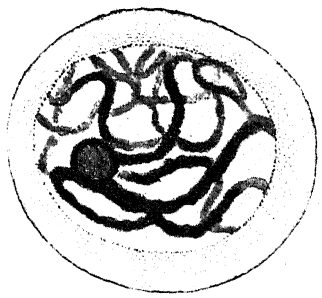

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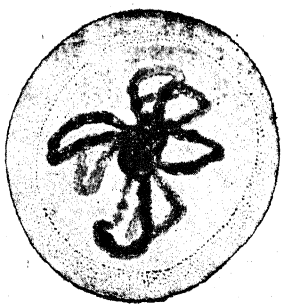

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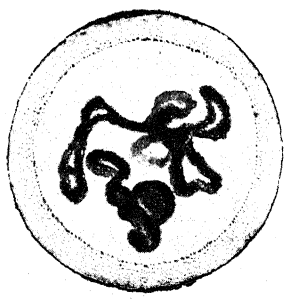

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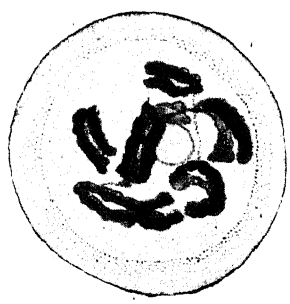

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The development of floral organs was also studied in *Corchorus capsularis* and it was observed that the sequence of development was the same as in *Corchorus olitorius*. The stamens as in "Chinsura Green" arise from the floral axis as protuberances. These grow upwards and become broad at the upper end. The upper elongated region becoming the anther and the lower slender base forms the filament.

Meiosis.

The young anther, which in transverse section is four-lobed in appearance, consists in early stages of its development of undifferentiated meristematic tissue. An archesporium is very soon developed in the hypodermal layer. This divides periclinally, and gives rise to a layer of primary sporogenous cells and a layer of parietal cells. The parietal cells divide by periclinal walls, and the cells thus formed divide in the same manner once again. The primary sporogenous layer divides later by periclinal or oblique walls, and as a result two layers of differentiated microspore mother cells are noted in the young anthers. The outer anther wall thus consists of an epidermal layer, two layers of elongated parietal cells, and an inner tapetal layer.

The tapetal layer after its differentiation is quite distinct in size and appearance. It contains a single nucleus in each cell which very nearly fills the entire cavity of the cell. During the activity of the microspore mother cells, the tapetal cells increase in size and their cytoplasm becomes vacuolate. Stages of mitotic divisions are not infrequently met with. Mitotic divisions of the tapetal nuclei have been noted by Dugger [1899] in *Bignoia venusta*, Maheswari [1929] in *Boerhaavia diffusa*, and also by other investigators. No cell plate is formed after the divisions, and the tapetal cell becomes binucleate. It should be mentioned, however, that all the tapetal cells do not become binucleate. Fragmentation of tapetal nuclei as described by O'Neal [1920] in *Datura stramonium* was not observed.

In the young anthers the sporogenous tissue is differentiated within the anther loculus. The cells are filled with finely granulated cytoplasm and are separated by delicate walls. They are generally polygonal in outline, there being no spaces between the individual cells and the cells of the tapetum. The resting nucleus of the microspore mother cells is nearly spherical in outline and is bounded by a hyaline membrane. The nucleolus generally lies in the centre of the nuclear cavity. A faintly staining reticulum consisting of fine chromatic granules is noted inside the nucleus (Plate XI, fig. 1). Usually a single nucleolus is present, but in some preparations two nucleoli equal in size, or one smaller than the other, have been noted. Preparations faintly stained with a view to study the nucleolar contents

showed that the peripheral region of the nucleolus had greater chromaticity than the central region, which thus appeared to resemble a vacuole (Plate XI, fig. 2). Crystal bodies as described by Latter [1926] in *Lathyrus odoratus* were not observed. Firky [1930] gives an account of the opinion held by various investigators on the origin and nature of these crystal bodies and he is inclined to believe that these are artefacts.

The activity of the nucleus on the onset of the meiotic prophase is seen in the reticulum which now resolves into definite threads and spreads throughout the nuclear cavity. The threads appear to be thick at some places and thin at others. General parallelism of the threads has not been observed, and it appears that the thick portions represent chance approximation of two threads. Granules of chromatin are scattered over the thread, being generally noted at the points of intersection. The nuclear cavity becomes very soon filled up with a dense tangle of leptotene threads which have the appearance of a continuous spireme. The position of the nucleolus is variable. As already mentioned, more than one nucleolus has been noted in a number of preparations. The nucleolus is enclosed by the reticulum and appears to be in contact with it (Plate XI, fig. 3). The shape of the nucleolus remains more or less spherical and its chromaticity is the same as before. No nucleolar budding, or extrusion of chromatin from the nucleolus as described by Digby [1909] in Galtonia was observed nor was any amœboid form of nucleolus as recorded by Wager [1904] and others found.

The onset of synizesis is noted by the characteristic changes in the reticulum. The threads at the periphery become somewhat thickened and the meshes become somewhat larger than those in the centre. The threads contract away from the periphery (Plate XI, fig. 4) and condense into a tight knot which lies adpressed to the nuclear wall, enclosing the nucleolus in its meshes (Plate XI, fig. 5). In some preparations it has been observed that while most of the threads were condensed to form the synizetic knot, one or more threads still remained attached to the periphery of the nuclear cavity in the form of loops. Careful examinations in most cases revealed that these loops were alveolized.

The contraction figure seen in synizesis has been suggested by some investigators to be an artefact due to the action of fixing fluids. Firky [1930] states "that the knot does not actually exist in nature and that its presence in fixed material is due to treatment". He also quotes Chodat's and McClung's views on the subject. The almost general presence of the knot, however, leads one to believe that it is an essential process in meiosis. The contraction as observed in fixed material is no doubt accentuated as a result of the action of the fixing fluid.

The synaptic knot next unravels itself and loops are thrown out in the nuclear cavity. The thread as it emerges from the synaptic knot appears to be thicker

than before and careful examination reveals that at places it is double. In most preparations the double nature of the thread could be followed for a short length only, but there could be no doubt as to its presence. It has the appearance of two threads having fused laterally (Plate XI, fig. 6). The spireme appears to be continuous and is looped considerably inside the nuclear cavity (Plate XI, fig. 7). The doubleness of the spireme may either indicate the reassociation of the split halves of the univalent chromosomes (Telosynapsis), or the pairing of entire lengths of univalent chromosomes (Parasynapsis). The evidence obtained during later stages of meiosis points to the conclusion that the spireme is univalent at this stage. As a result of the unravelling of the synaptic knot a typical open spireme is formed which fills the nuclear cavity (Plate XI, fig. 8). The nucleolus appears to be somewhat pale but its form appears to be the same as before. It generally lies in the centre of the nuclear cavity.

The spireme next thickens and contracts, and appears to be more homogeneous than before. All traces of the longitudinal split in the spireme are obscured at this stage and it appears as a thick coiled rope lying free in the nuclear cavity. Considerable portions of the spireme could be traced successfully at this stage. This stage has generally been reckoned as the "Pachynema" stage and appears to be of very short duration.

The first signs of the separation of the pollen mother cells are noted at this stage. Clear spaces appear between the individual cells but they still maintain their angular outline. The process of 'rounding off' takes place immediately afterwards, and from the next stage onwards the pollen mother cells lie free in the microsporangium.

The deep staining pachytene thread which fills the nuclear cavity (Plate XI, fig. 9) now shows signs of contraction by drawing in of the loops towards the centre of the nucleus. The spireme, as has been indicated before, is a continuous structure and does not show any longitudinal split. It forms a loose knot of tangled threads in the centre of the nuclear cavity with the nucleolus situated close to it. Loops or strands escape from the central mass and are spread out in the nuclear cavity. Figure 10 in Plate XI shows a favourable preparation where the number of radiating loops is seen to be seven, which corresponds with the haploid chromosome number of the plant. The nucleolus is situated centrally, which thus obscures the orientation of the loops in that region, but so far as could be made out from the examination of similar preparations, the spireme appears to be single and the loops appear to be continuous at this stage. The arrangement of the spireme indicates that chromosome conjugation in this plant takes place telosynaptically, as indicated by Farmer and Moore [1905]. The adjacent portions of the spireme are composed of two homologous chromosomes arranged '*in*

tandem'. Since the spireme was seen to be a continuous filament the fourteen chromosomes were evidently arranged end to end. The orientation of the seven loops and their subsequent twisting and segmentation at the central region of the contracted knot appears to support the telosynaptic theory of the origin of the bivalent chromosomes. The split in the looping stage obviously represents the separation plane of the two halves of the univalent chromosomes. The loops then undergo further condensation and contraction and the two arms of a loop (*i.e.*, a pair of homologous chromosomes) become twisted round each other, or are intimately associated with each other (Plate XI, fig. 11). Segmentation of the spireme into pairs of chromosomes immediately follows, and at this stage it is possible to make out the seven pairs of chromosomes (Plate XI, figs. 11 and 12).

The bivalent chromosomes thus formed remain united end to end before undergoing further condensation (Plate XI, fig. 12). The two arms of a bivalent pair are arranged in various ways and not infrequently take the forms of X, O, V or 8. In some cases the twisting of the chromosomes is so great that they appear as univalents. The seven pairs of bivalent chromosomes noted in early diakinesis show that they are in different stages of condensation. Their irregular outline is quite evident, and in most preparations the seven pairs could be seen scattered in the nuclear cavity. Condensation of the chromosomes proceeds rapidly and the chromosomes very soon assume a compact form and the plane of separation of the univalents becomes indistinguishable. This may also be due to imperfect fixation. The chromosomes in late diakinesis are rod-shaped (Plate XII, fig. 13), and are scattered in the nuclear cavity which is bounded by a distinct membrane. In no case, either in early or late diakinesis, was a split observed in the homologous chromosomes. The nucleolus at this stage maintains its spherical outline, but is very pale in appearance (Plate XII, fig. 13). It should be noted, that of the seven bivalent chromosomes one pair was observed to be slightly longer than the others. It was not possible, however, to trace this difference in size from heterotypic metaphase onwards.

In late diakinesis the nuclear membrane gradually disappears, its edges first becoming obscure. The development of the spindle fibres which appear to be intranuclear in origin was, however, not studied in detail. No multipolar spindle was observed. This might be due to the effect of the fixing fluid employed. Latter [1926] mentions that Allen's modified "Bouin's fluid was not satisfactory for the study of nuclei at this period, no trace of spindles prior to the definite bipolar form being found, though the general fixation was good".

In the metaphase of the heterotypic division the chromosomes are arranged on the equatorial region of the spindle and in well-fixed preparations their dyad nature is quite evident (Plate XII, fig. 15). The spindle is quite sharp and pointed

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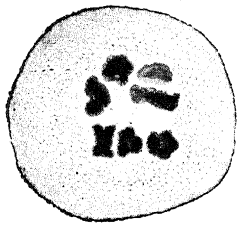

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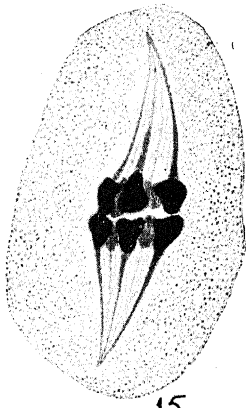

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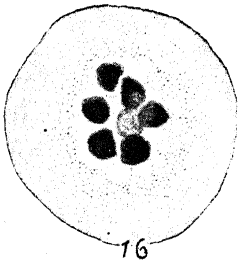

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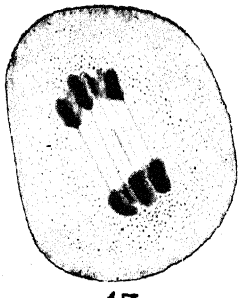

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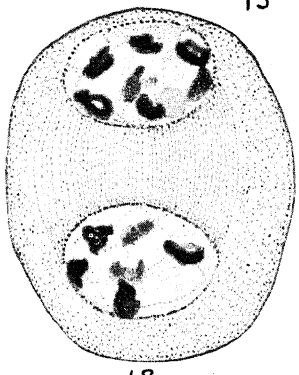

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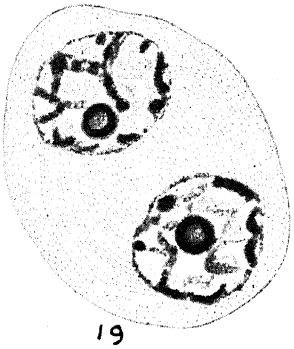

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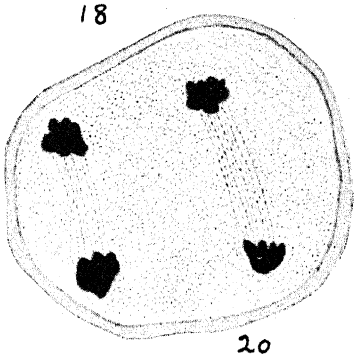

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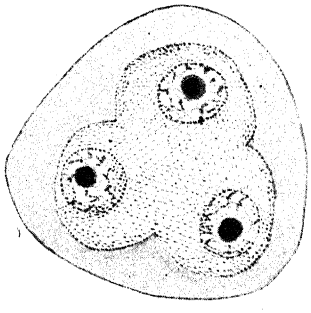

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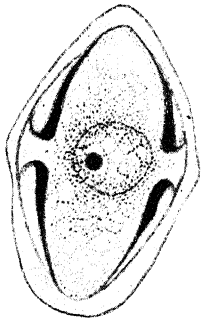

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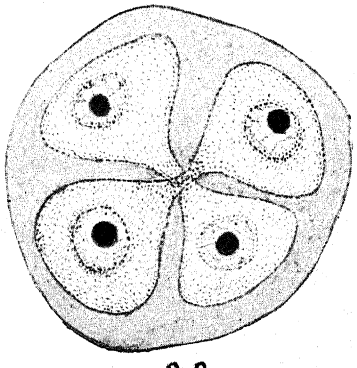

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at both ends. A few strands of fibres are seen attached to the chromosomes. At this stage the nuclear area is not distinguishable from the surrounding cytoplasm which appears to be comparatively dense. No perinuclear zone, as observed by Denham [1924], in cotton was noted, neither was any general clumping of chromosomes on the equatorial plate observed. Polar view of an equatorial plate at this stage shows seven bivalent chromosomes (Plate XII, fig. 16). The chromosomes are very much condensed and appear to be all alike.

In the anaphase the chromosomes appear to be pulled apart by strands of fibres which are attached to their ends. In the early stages of the separation of the univalents, they seem to be attached to each other by bond like projections, which, however, are soon absorbed. The chromosomes at first assume an irregular outline, but soon begin to round off. The chromosomes as they move towards the poles are independent of each other, their movement is quite regular and in no case was any 'lagging' chromosome or 'non-disjunction' noted. On reaching the poles the chromosomes clump together and their individuality is lost sight of (Plate XII, fig. 17). The spindle fibres at this stage are stretched from pole to pole and are perfectly straight.

Figure 18 in Plate XII represents an interkinetic stage where the chromosomes appear to be free from one another and are spread in the nuclear cavity. The homotypic split appears suddenly and is evident in most of the chromosomes at this stage, and some of them are arranged in the form of V. In a number of preparations the seven chromosomes which lie scattered in the nuclear cavity could be easily made out. The chromosomes at first appear to be somewhat angular in outline, but very soon they elongate and appear to be connected with each other by delicate strands. A nuclear wall is developed at this stage and a small nucleolus is also noted in each nucleus (Plate XII, fig. 19).

While the nuclei are being reorganised, the spindle fibres disappear and are replaced by granular striations in the cytoplasm, giving the protoplast a barrel-shaped appearance. The nuclei remain in the interkinetic stage for some time before it passes on to the second divisional stages.

In the metaphase of the homotypic division the chromosomes are grouped together in the equatorial region of the spindle. They appear to be smaller than those observed in the first division. There is no difference in shape or size between the individual chromosomes. The movement of the chromosomes to the poles is quite regular. Stringing out of chromatin in fibrils, or lagging chromosomes, were not observed. On reaching the poles the chromosomes clump together (Plate XII, fig. 20) and their identity is lost for a while, but very soon they move apart and pass on to the telophasic stage. The spindle fibres become obliterated and

the chromosomes are distinct from each other at this stage, and appear to be spread in a hyaline sap. In favourable preparations it is possible to count the seven chromosomes. Very soon the nuclei are organised with a nuclear membrane and a deep staining nucleolus. The chromosomes next elongate slightly and are connected to one another by faintly staining strands. The nuclei then pass on to the resting condition and remain in that state for a relatively long time.

The space relationships of the homotypic spindles are very variable. They are usually parallel to each other (Plate XII, fig. 20), but occasionally they have been observed to lie at right angles or at acute angles. Whatever their arrangement might be, a tetrahedral arrangement of four microspores is always met with. In no case was a bilateral arrangement of the microspores observed.

When the reduction divisions are completed the microspores secrete a mucilaginous pellicle which surrounds the tetrad and completely envelops it. Cytokinesis is brought about by furrowing. The process has been fully described by Farr [1916], and Reeves [1930] has also given an account of the various views on the subject, which need not be repeated here. The cleavage furrows were first noted after the tetrads were formed and not after the heterotypic division as has been mentioned by some investigators. The furrows first appeared at the periphery of the cytoplasm at four points, equidistant to one another (Plate XII, fig. 21). The constrictions gradually cut in the cytoplasm and meet in the centre of the cell (Plate XII, fig. 22). The protoplast thus separates into four cells. The mucilaginous layer extends into the furrows and forms plates between the separating microspores, which appear to be embedded in a sphere of hyaline material. The young microspores after a period of rest are liberated into the microsporangium by the dissolution of the mucilaginous membrane. Each microspore is now surrounded by a cell wall which probably results from the thickening of the previous cell membrane.

The cytoplasm of the liberated microspore appears to be somewhat dense and it contains a spherical nucleus in each of which a single nucleolus and chromatin granules are noted. At this stage the microspores rapidly increase in size and the outer walls become differentiated. Three sulci are noted on the wall and the germpores are situated centrally one in each of these sulci. The walls of the pollen grains are folded inwards along the sulci, and as a result the pollen grains appear oval in outline. When the pollen grains become turgid the walls expand, and the microspores appear round in form. A single nucleus embedded in a mass of cytoplasm and situated centrally is noted in the young pollen grain (Plate XII, fig. 23). No nuclear division was noted inside the pollen grain, nor was any pollen grain found to be binucleate.

SUMMARY.

The development of the flower and pollen has been studied in *Corchorus olitorius*.—(' Chinsura Green ').

1. The development of the floral organs in *Corchorus olitorius* and also in *Corchorus capsularis* is centripetal. The sequence of development being, sepals, petals, stamens and pistil. The growth of the corolla is arrested at an early stage of its development. It, however, resumes growth when the other floral members have been differenciated.

2. The resting nucleus of the microspore mother cells has a granular reticulum. The nucleolus generally occupies a central position.

3. A very contracted ' knot ' is noted in synizesis. The knot lies at one side of the nuclear cavity adpressed to the nuclear wall. The nucleolus is entangled in the meshes of the knot.

4. A second contraction stage is noted and the method of chromosome conjugation is telosynaptic. Seven loops have been counted at this stage. Each loop is composed of two homologous chromosomes united at the apex of the loop.

5. In diakinesis the two members of a bivalent chromosome, lie side by side or are arranged in various ways. The homotypic split was not observed at this stage.

6. The heterotypic and homotypic divisions are regular.

7. An interkinetic stage of some duration is passed through before the commencement of the second division. The nuclei are well organised and the homotypic split is noted in most of the chromosomes at this stage.

8. Cytokinesis takes place by furrowing.

9. The young pollen grains contain a single nucleus situated centrally.

10. The haploid number of chromosomes is seven, the diploid fourteen.

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Explanations of plates.

All the figures were drawn with a camera lucida and have been reproduced without reduction. The figures were drawn under 1/12 a inch imm., Leitz N. A. 1·32 with compensating ocular 12. Figs. 17 and 23 were drawn under 1/12 a inch imm., Leitz N. A. 1·32 with ocular 10.

Plate XI.

Fig. 1. Resting nucleus of pollen mother cell. The reticulum has a granular appearance.
Fig. 2. Prophase. Leptonema threads well formed.
Fig. 3. Prophase. Leptonema threads show first signs of contraction. Note alveolisation at certain regions of the spireme.
Fig. 4. Beginning of first contraction. The nuclear contents are withdrawn from the periphery and are aggregating in the centre irregularly.
Fig. 5. First contraction—the nucleolus enclosed by the synaptic knot.
Fig. 6. The spireme is emerging from synapsis in the form of loops. Some of the outer bends of the loops show distinct fission.
Fig. 7. Early hollow spireme. The split in the spireme is still evident at certain places.
Fig. 8. Hollow spireme.
Fig. 9. Pachynema stage.
Fig. 10. Second contraction. Seven loops are seen to radiate from the centre of the nucleus.
Fig. 11. Segmentation of the seven loops to form the bivalent chromosomes is noted. The two arms of a loop are twisted round each other.
Fig. 12. Early Diakinesis—the seven bivalent chromosomes are scattered in the nuclear cavity. The nucleolus is very pale in appearance.

Plate XII.

Fig. 13. Diakinesis—one bivalent pair appears to be slightly longer than the others.
Fig. 14. Chromosomes aggregating towards the equatorial plate.
Fig. 15. Heterotypic metaphase.
Fig. 16. Polar view of an equatorial plate showing the haploid number of chromosomes (seven).
Fig. 17. Telophase of the first division.
Fig. 18. Interkinesis—note split in the univalents.
Fig. 19. Interkinesis—the chromosomes have become stretched out and anastomoses are noted. Nucleolus also is seen at this stage.
Fig. 20. Homotypic division—telophase.
Fig. 21. Quadripartition of mother cell protoplast by furrowing.
Fig. 22. Pollen tetrad. Microspores enclosed in a mucilaginous pellicle and are not completely separated as yet.
Fig. 23. A young microspore, with a single nucleus placed centrally.

THE F_1 GENERATION OF A HYBRID BETWEEN AN AMERICAN AND AN ASIATIC COTTON.*

BY

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(Received for publication on 7th November 1932)

(With Plates XIII and XIV)

It is well known that crosses between Asiatic and American cottons are difficult on account of the differing number of their chromosomes, thirteen being the haploid number in the former and twenty-six in the latter. Till now only few cases of such crosses have come to the notice of the writer, the first reported by Zaitzev [1922-23], the parents of which were *Gossypium herbaceum* (female) and *G. hirsutum* (male), the second of similar parentage by Desai [1927], and the third, a recent one (*G. barbadense* × *G. arboreum*) by Harland [1932]. Harland [*Loc. cit.*] refers to two more recent cases, one (*G. herbaceum* × *G. hirsutum*) reported by Vycotski and the other in which the New World type has been used as female by Nakatomi.

The result obtained by the workers cited here and of the writer himself with the exception of Harland has been that the hybrid of a cross between the old and new world cotton has hitherto proved sterile. Harland's work, therefore, in obtaining a hybrid which although sterile on the female side has proved to possess some functional pollen grains which were used for back crossing with *G. barbadense* is note-worthy in that it shows that successful crosses giving a fertile or partially fertile hybrid are still possible between the Old and New World cottons. From the seeds of back crosses Harland has successfully raised a few F_2 plants which from their characters seem to indicate as having derived a few chromosomes of the Asiatic parent. Back crossed plants when again crossed with *G. barbadense* have proved to be fertile on both female and male sides which thus leads to the possibility of evolving a new race of cotton combining in itself the desirable qualities of the Old and New World types.

The present note deals with the description of the first generation hybrid between an American and an Asiatic cotton and adds to, and in a way confirms, the experience of previous workers. In the year 1928, the writer had made an attempt to fertilize about fifty emasculated flowers of Bourbon (*G. purpurascens*) with pollen

* The author is indebted to Dr. W. Burns, Economic Botanist to the Government of Bombay, for help in writing this paper.

of Wagad 12 (*G. herbaceum*) and other strains of Wagad. No special technique was resorted to except the following :—

The anthers of Bourbon flowers were found to burst earlier by about three hours, *i. e.*, at about 8 A. M. than those of Wagad 12 and other Wagad strains. Thus it seemed that the stigmas of Bourbon would be receptive comparatively earlier. In order to make the anthers of Wagad 12 and other Wagad strains burst earlier the writer used to remove the corolla of flowers and keep the androecium exposed to the sun, taking precautions that the pollen grains did not get contaminated with any other pollen. It was thus possible to apply the pollen to the emasculated flowers of Bourbon at about 10 A. M. With the exception of one flower pollinated with the pollen of Wagad 12, the rest of the cross-pollinated flowers dropped. This flower gave a boll with a single seed in it. The peculiarity of Bourbon is that some of its bolls develop even with a single fertilized ovule, while in most of the other cottons, even bolls with two or three fertilized ovules shed. This seed was sown with special care at the Cotton Breeding Station, Viramgam, in the month of June 1929. The plant grew successfully and the various characters interesting from the point of view of its parentage were observed. The mode of inheritance of each character has been described below :—

(*1*) *Purple leaf spot.*

Bourbon.—Purple leaf spot is present near the pulvinus both in cotyledons and other leaves.

Wagad 12.—Spot quite absent.

Hybrid.—Spot absent in cotyledons. It appeared as a clear red spot in young leaves. As the plant grew older, there was no visible difference between the purple leaf spot found in Bourbon and the one found in the leaves of the hybrid.

(*2*) *Purple coloration of branches and stem.*

Bourbon.—Purple color throughout except on young shoots.

Wagad 12.—Absence of purple coloration of the Bourbon type.

Hybrid.—Purple coloration as in Bourbon.

(*3*) *Hairiness of the stem and leaves.*

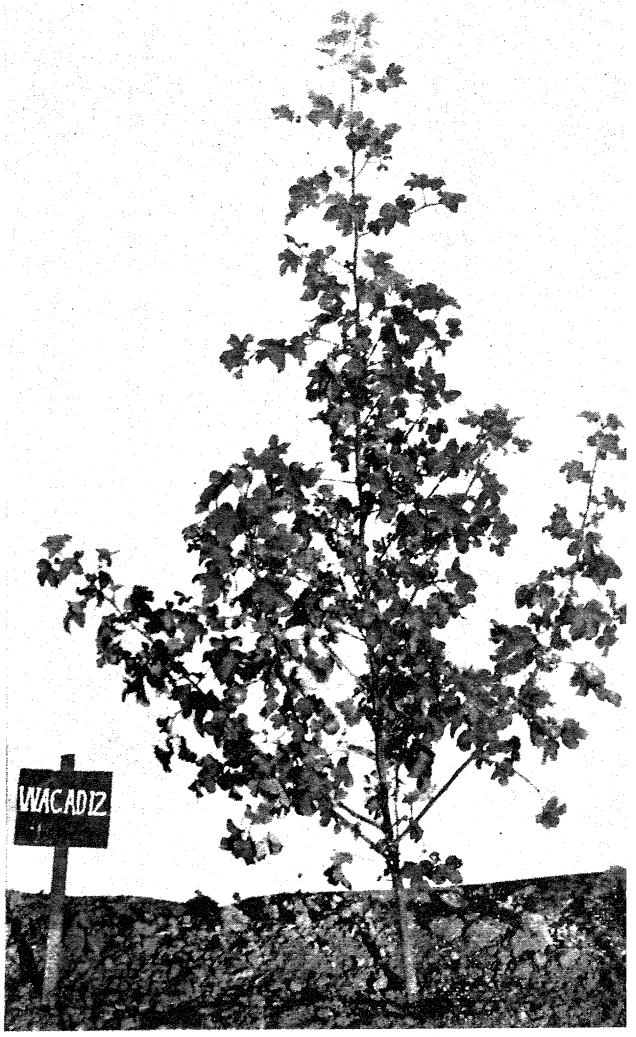
Bourbon.—Almost glabrous.

Wagad 12.—Hairy.

Hybrid.—Intermediate but more like the glabrous parent.

Bourbon.

Bourbon × Wagad 12, F_1.



Wagad 12.

(*4*) *Height* (*Plate XIII*).

Bourbon.—Perennial—growth being not more than one to one and a quarter foot in the first year. Growth very quick during later generations, the final height being fifteen to twenty feet.

Wagad 12.—Annual. Two and a half to three feet high.

Hybrid.—Intermediate between perennial and annual habit. Growth not quick in the second year. Height intermediate but showing partial dominance of tallness.

(*5*) *Position of first sympodium on the main stem.*

Bourbon.—First sympodium at a high node, *i.e.*, eighteenth node and above.

Wagad 12.—First sympodium at a low node, *i.e.*, fourteenth node on an average.

Hybrid.—Dominance of lower node. Actual node number was twelve.

(*6*) *Flowering habit.*

Bourbon.—Flowering later than Wagad 12 by fifteen to twenty days.

Wagad 12.—Early.

Hybrid.—Flowering as found in Wagad 12 and hence dominance of early flowering.

(*7*) *Leaves* (*Plate XIV, fig. 1*).

Bourbon.—Leaves broad and big with three shallow lobes. Sinus between the lobes not thrown up in folds.

Wagad 12.—Leaves small with five lobes deeply cut. Sinus thrown up in folds.

Hybrid.—Leaves broad as in Bourbon with five lobes as found in Wagad 12. Lobes intermediate, *i. e.*, neither deep as in Wagad 12 nor shallow as in Bourbon. Sinus thrown up in folds as in Wagad 12.

(*8*) *Dentation of bracteoles* (*Plate XIV, fig. 2, upper row*).

Bourbon.—Long teeth.

Wagad 12.—Very small teeth.

Hybrid.—Dominance of long teeth.

(*9*) *Petal spot* (*Plate XIV, fig. 2, lower row*).

Bourbon.—No spot.

Wagad 12.—Big purple spot.

Hybrid.—Spot intermediate; actually it was on the small side.

(*10*) *Petal colour.*

Bourbon.—Very pale yellow.
Wagad 12.—Yellow (full).
Hybrid.—Yellow (full) and hence dominance of yellow (full).

(*11*) *Pollen colour.*

Bourbon.—White.
Wagad 12.—Yellow (full).
Hybrid.—Yellow (full) and hence dominance of yellow (full).

(*12*) *Position of stigma in the bud stage.*

Bourbon.—Stigma visible in the bud stage as petals at the top do not overlap completely.

Wagad 12.—Stigma not visible in the bud stage as petals at the top completely overlap each other.

Hybrid.—Same as found in Bourbon.

The hybrid (Bourbon × Wagad 12) was quite sterile, though the flowering was profuse. The anthers were found shrivelled up. Back-crossing with the pollen of either parent was tried but with no success.

In two respects the writer's results have agreed with Harland's, firstly considering the few crosses made the success could be attributed to having used the American as the female parent, and secondly the single seed produced in a boll has been observed by Harland as usually occurring.

It was proposed to make chromosome studies of the hybrid but the plant was accidentally destroyed in 1931.

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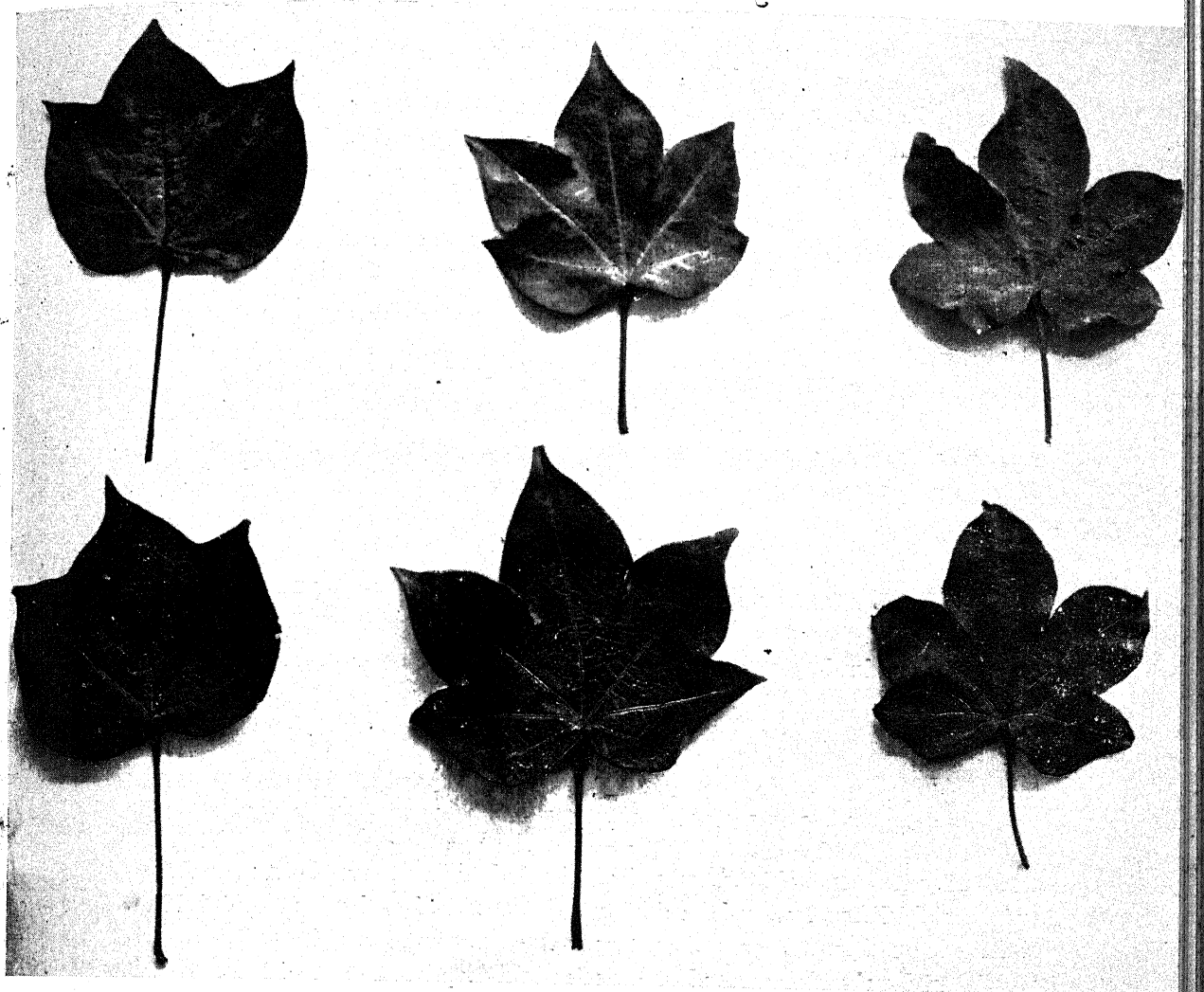
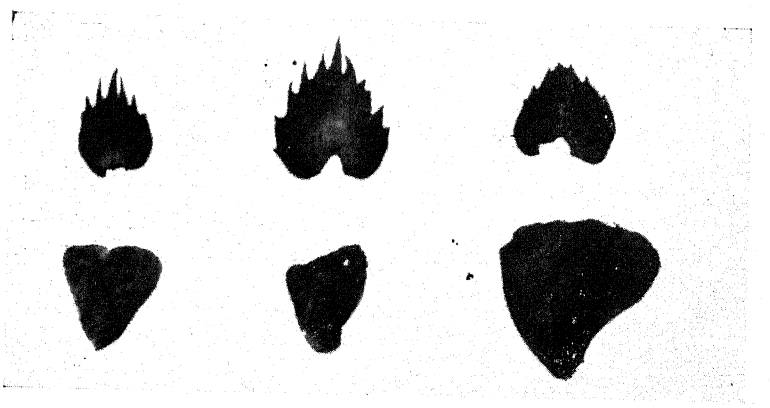


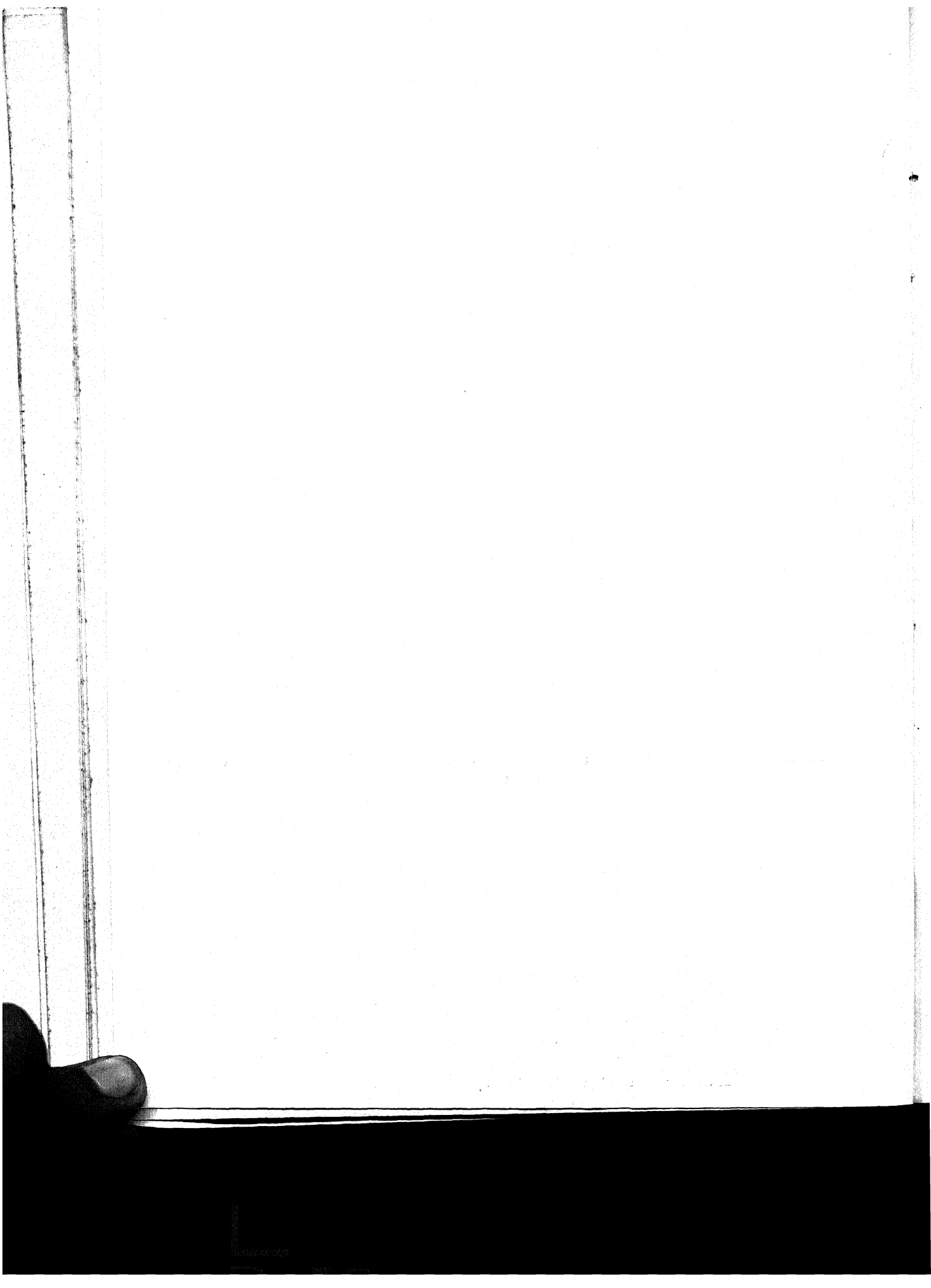
Bourbon. Bourbon × Wagad, F_1. Wagad 12.

Fig. 1.



Bourbon. Bourbon × Wagad 12, F_1. Wagad 12.

Fig. 2.



STATISTICAL NOTES FOR AGRICULTURAL WORKERS.*

No. 6.—THE EFFECT OF FERTILIZERS ON THE VARIABILITY OF THE YIELD AND THE RATE OF SHEDDING OF BUDS, FLOWERS AND BOLLS IN THE COTTON PLANT IN SURAT.

BY

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(Received for publication on 3rd October 1932).

1. Mr. K. V. Joshi of the Cotton Research Laboratory, Surat, has sent us for statistical analysis the results of certain experiments † conducted by him in 1930-31 with fertilizers on selected strains of the cotton plant.

The effect of fertilizers may be divided into two distinct groups—(A) changes in the mean value of the yield, or the mean rate of shedding at different stages, and (B) changes in the variability of the yield or the variability of the rate of shedding. Consider the production of "buds". The application of fertilizers may affect the number of "buds" produced. It may also affect the variability of the bud production from plant to plant under the same treatment. These two effects must be carefully distinguished.

Fisher's method of "analysis of variance" is designed to test whether the mean values are affected or not, that is, to investigate effect (A). In this method it is assumed that the variabilities are identical, *i.e.*, that effect (B) is inappreciable.

It is quite possible, however, that mean values are not affected, *i.e.*, effect (A) is inappreciable, while effect (B) is not negligible, so that variabilities are appreciably altered.

Speaking generally it is desirable to investigate both the effects. In case variabilities are appreciably the same (*i.e.*, effect (B) is neglible), Fisher's z-test can be applied to test whether mean values have altered. If effect (B) is not negligible, further studies may become necessary. Neyman and Pearson [1931] have considered this problem very fully in a recent paper and have developed suitable methods for disentangling the different effects. I shall use Mr. Joshi's data to illustrate the use of such methods.

* We are receiving a large number of enquiries of a statistical nature from agricultural workers in different parts of India. Many of these enquiries are of cosiderable general interest, and it is proposed to publish notes on selected topics from time to time. These notes will deal mainly with statiscal methods and procedure, and it is not intended that they should always contain new matter.—Ed.

† The actual data will be found in Appendix I given at the end of the paper and fuller details in another paper, Statistical Notes No. 7. This Journal, Vol. 3, p. 139.

2. Neyman and Pearson [1931] have distinguished three different cases :—

(*i*) The hypothesis H_0 that the samples belong to populations having the same mean value and the same variance, so that so far as the mean value and the variance are concerned, the samples may be considered to have come from the same population [1930].

(*ii*) The hypothesis H_1 that the samples come from populations having the same variance. The mean value may be different for each population (or identical as a special case).

(*iii*) The hypothesis H_2 that the mean values are identical, it being assumed that the variances are also identical.

It will be noticed that (*i*) H_0 will test whether both mean values and variances are identical, (*ii*) H_1 whether variances are equal or not, and (*iii*) H_2 whether mean values are equal or not, it being assumed that variances are identical. Test of H_2 is simply an alternative form of Fisher's *z*-test and need not be further considered here. It must be emphasized, however, that Fisher's method of analysis of variance in its usual form can only be applied on the assumption that the variances are identical.

3. It will be now necessary to define certain statistical parameters.

Let n_t, m_t and S_t^2 be the size, mean value and variance of the "t"th sample. Then

$$n_t m_t = S(x) \qquad n_t s_t^2 = S(x - m_t)^2 \quad . \quad . \quad . \quad . \quad (1)$$

where S represents a summation for all n_t values of x for the "t"th sample. s_t^2 is thus the observed standard deviation for a single sample. Let there be k such samples.

The average variance s_a^2 within all samples is defined by :—

$$N = \Sigma(n_t) \qquad N s_a^2 = \Sigma(n_t s_t^2) \quad . \quad . \quad . \quad . \quad (2)$$

where Σ represents a summation for all "k" samples, and N is the total number of individual observations available.

Finally the general mean m_0 and the general variance s_0^2 are defined by

$$N m_0 = \Sigma S(x), \quad N s_0^2 = \Sigma S(x - m_0)^2$$

where ΣS represents the summation for all N values of x. It will be noticed that s_0^2 is the "total" variance, and s_a^2 the mean variance "withins amples" ordinarily used in the analysis of variance.

A case of special importance occurs when the size of the sample is the same for all samples, *i.e.*, $n_1 = n_2 = \ldots\ldots\ldots n_t = n$, and $N = nk$. In this case there is a considerable simplification in the working formulae. It is therefore extremely desirable to arrange the size of the sample to be the same, whenever this is possible, in field experiments.

* The author has considered this problem in greater detail from a theoretical standpoint in a different paper [1930].

Neyman and Pearson's formulae can then be put in the following form.

$$l_0=\left(\frac{s_1^2}{s_0^2}\cdot\frac{s_2^2}{s_0^2}\cdot\ \ .\ \ .\ \text{upto}\ \frac{s_k^2}{s_0^2}\right)^{1/k} \quad . \quad . \quad . \quad . \quad . \quad (4)$$

$$l_1=\left(\frac{s_1^2}{s_a^2}\cdot\frac{s_2^2}{s_a^2}\cdot\ \ .\ \ .\ \text{upto}\ \frac{s_k^2}{s_a^2}\right)^{1/k} \quad . \quad . \quad . \quad . \quad . \quad (5)$$

We may introduce the geometric mean of the variances defined by :—

$$s_g^2=(s_1^2.\ s_2^2\ \ .\ \ \text{upto}\ s_k^2)^{1/k} \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

or its logarithmic form :—

$$\log s_g^2=1/k\ (\log s_1^2+\log s_2^2+\ \ .\ \ .\ \ +\log s_k^2) \quad . \quad . \quad . \quad (6{\cdot}1)$$

Then $\log l_0=\log (s_g^2)-\log (s_0^2)$ . . . . . (4·1)

$$\log l_1=\log (s_g^2)-\log (s_a^2) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5{\cdot}1)$$

Also $\log l_2=\log (s_a^2)-\log (s_0^2)$ . . . . . (7)

So that $\log l_0=\log l_1+\log l_2$ . . . . . (8)

The formulae for the distribution of l_0 and l_1 have been given by Neyman and Pearson [1931].

4. The interpretation of l_0 and l_1 is extremely simple. If hypothesis H_0 is true (that is, if all "k" samples are drawn from a population having the same mean value and the same variance) then l_0 will be sensibly equal to unity. In the same way if hypothesis H_1 is true (that is, if the "k" samples are drawn from populations having the same variance but with either the same or different mean values), then l_1 will be sensibly equal to unity. On the other hand as l_0 and l_1 become smaller and smaller the hypothesis H_0 and H_1 respectively become less and less probable. In other words if l_0 is found to be significantly less than unity, the observed samples cannot be considered to have come from the same population. In the same way if l_1 is found to be sensibly lower than unity then the observed samples must be considered to belong to populations having different variabilities.

With the help of the formulae for moment co-efficients, given by Neyman and Pearson, it is possible to calculate the 5 per cent. and one per cent. points for both l_0 and l_1, and hence judge whether l_0 or l_1 is significantly lower than unity, or may be considered sensibly equal to unity.

The statistics η_2 is equal to $(1-\eta^2)$ where η is the "correlation ratio" of Karl Pearson. When l_2 is small η^2 is large, so that the mean values for the different samples cannot be considered to be identical; l_2 thus furnishes simply an alternative form of Fisher's z-test.

It will be noticed that $l_0=l_1.\ l_2$, so that the value of l_0 may be reduced either due to l_1 or l_2. Thus hypothesis H_0 may become improbable owing to (*i*) the variabilities being different, or (*ii*) the mean values being different, or (*iii*) due to the joint effect of both the factors.

5. I shall now consider Mr. Joshi's data. The "control" plot will be referred to as sample No. 1, the July-manured plot as sample No. 2, and the August-manured plot as sample No. 3.

The calculation of l_0, l_1 and l_3 (or z) is quite simple and straightforward. The variances s_1^2, s_2^2, s^2 for the three samples are determined directly and the weighted geometric mean s_g^2 is calculated with the help of logarithms. The mean variance within samples s_a^2, and the general variance s_0^2 are required for the z-test and are calculated in the usual way.

The calculation for the number of "buds" is shown in Table (I,1). Adding the logarithms of the three individual variances, we get 11·9097214. Dividing by $k = 3$ we get the weighted geometric mean log s_g^2= 3·9699071. Subtracting the logarithm of s_a^2 (=4·0134755) from logs_2^2, we get logl_1=1·9564316. Similarly subtracting logs_0^2 (=4·1392424) we get log l_0=1·8306647. The observed value of l_0=0·8741 and of l_1=0·9465. (For comparison we also find that l_2=0·9236.)

The variances and calculations for the number of "flowers", "bolls" and the proportion of "flowers : buds", "bolls : flowers" and "bolls : buds" are shown in Tables (I, 2)—(I, 6).

The observed values of l_0, l_1, l_2 and z are shown in Table II.

6. We can now use Neyman and Pearson's theory to judge the significance of these observed values of l. Using the formulae for moment co-efficients given by them, we find the following values for the 5 per cent. and one per cent. points of l_0 and l_1 for $n = 20$, $k = 3$. The 5 per cent. and one per cent. values of z are also given for comparison.

Size of sample		Level of significance	l_0	l_1	z
$n = 20$, $k = 3$	$N = n \cdot k = 60$	5 per cent.	0·8417	0·8912	0·5764
		1 per cent.	0·7816	0·8334	0·8065

The significance of the expected values is clear. If hypothesis H_0 were true, that is, if sets of 3 samples of 20 ($k = 3$, $n = 20$) were repeatedly drawn from the same normal population, then the observed value of l_0 would be less than 0·8417 in 5 per cent. and less than 0·7816 in one per cent. of cases. Similarly if hypothesis H_1 were true, that is, if sets of 3 samples of 20 were drawn from normal populations with an identical variability (but equal or different mean values), then the observed value of l_1 would be less than 0·8912 in 5 per cent. and less than 0·8334 in one per cent. cases.

TABLE (I, 1).
Buds.

Sample	Variance	log
s_1^2 . . .	1,69,00·25	4·2278932
s_2^2 . . .	70,77·30	3·8498676
s_3^2 . . .	67,91·42	3·8319606
Total .		11·9097214
s_g^2 . . .	Average .	3·9699071
s_a^2 . . .	1,03,15·15	4·0134755
s_0^2 . . .	1,37,79·78	4·1392424
l_0 . . .	s_g^2/s_0^2	$\bar{1}$·8306647
l_1 . . .	s_g^2/s_a^2	$\bar{1}$·9564316
l_2 . . .	s_a^2/s_0^2	$\bar{1}$·8742331

TABLE (I, 2).
Flowers.

Sample	Variance	log
s_1^2 . . .	14,66·35	3·1662376
s_2^2 . . .	1,10,74·05	4·0443065
s_3^2 . . .	13,75·89	3·1385838
Total .		10·3491279
s_g^2 . . .	..	3·4497093
s_a^2 . . .	46,91·07	3·6715496
s_0^2 . . .	49,55·73	3·6950501
l_0 . . .	s_g^2/s_0^2	$\bar{1}$·7546592
l_1 . . .	s_g^2/s_a^2	$\bar{1}$·7781597
l_2 . . .	s_a^2/s_0^2	$\bar{1}$·9764995

TABLE (I, 3).
Bolls.

Sample	Variance	log
s_1^2 . . .	1,29·01	2·1106234
s_2^2 . . .	77·39	1·8886848
s_3^2 . . .	1,04·28	2·0182010
Total .		6·0175092
s_g^2 . . .	..	2·0058364
s_a^2 . . .	1,03·55	2·0151501
s_0^2 . . .	1,15·15	2·0612631
l_0 . . .	..	$\bar{1}$·9445733
l_1 . . .	..	$\bar{1}$·9906863
l_2 . . .	..	$\bar{1}$·9538870

TABLE (I, 4).
Flowers : Buds.

Sample	Variance	log
s_1^2 . . .	1,04·65	2·0197392
s_2^2 . . .	1,09·65	2·0400086
s_3^2 . . .	39·26	1·5939503
Total .		5·6536981
s_g^2 . . .	..	1·8845660
s_a^2 . . .	85·29	1·9308981
s_0^2 . . .	101·43	2·0061664
l_0 . . .	..	$\bar{1}$·8783996
l_1 . . .	..	$\bar{1}$·9536679
l_2 . . .	..	$\bar{1}$·9247317

TABLE (I, 5).
Bolls : Flowers.

Sample	Variance	log s^2
s_1^2 . . .	13·00	1·1139434
s_2^2 . . .	26·05	1·4158077
s_3^2 . . .	29·83	1·4746533
Total .		4·0044044
s_g^2 . . .	..	1·3348015
s_a^2 . . .	..	1·3586961
s_0^2 . . .	..	1·3932241
l_0 . . .	..	1·9415774
l_1 . . .	..	1·9761054
l_2 . . .	..	1·9654720

(TABLE I, 6).
Bolls : Buds.

Sample	Variance	log s^2
s_1^2 . . .	8·79	0·9439889
s_2^2 . . .	11·09	1·0449315
s_3^2 . . .	4·53	0·6560982
Total .		2·6450186
s_g^2 . . .	..	·8816729
s_a^2 . . .	8·20	0·9138139
s_0^2 . . .	10·03	1·0013009
l_0 . . .	..	1·8803720
l_1 . . .	..	1·9678590
l_2 . . .	..	1·9125130

TABLE II.
Observed values of l_0, l_1, l_2 *and* z.

Character	l_0	l_1	l_2	z
Buds	0·6771	0·9046	0·7486	1·1207
Flowers	0·5684	0·6000	0·9473	0·2215
Bolls	0·8802	0·9788	0·8993	0·5718
Flowers : Buds	0·7558	0·8988	0·8409	0·8250
Bolls : Flowers	0·8741	0·9465	0·9236	0·4185
Bolls : Buds	0·7579	0·9287	0·8176	0·9179
5 per cent.	0·8417	0·8912	0·8999	0·5764
One per cent.	0·7816	0·8334	0·8503	0·8065

7. Adopting an one per cent. level of significance we notice that l_0 is significantly lower than unity in 4 cases : (i) Buds, (ii) Flowers, (iii) Ratio of flowers to buds, and (iv) Ratio of bolls to buds, showing that the application of fertilizers produces significant effects in the case of "buds", "flowers", and the ratio of shedding of buds.

With the help of l_1 and l_2 (or rather z), we can make a deeper analysis. The z-test shows that the mean values are significantly different in the case of (i) "buds", and the ratio of (ii) "flowers : buds" and (iii) "bolls : buds", while l_1 shows that the variabilities are different in the case of "flowers". In the case of "flowers : buds" the l_1-test is on the verge of significance.

We conclude that the application of fertilizers has had the following effects—

(*i*) The mean number of "buds", the mean proportion of "flowers : buds", and the mean proportion of "bolls : buds" are all altered appreciably. The effect on the production of "buds" is apparently the basic factor. The application of Fisher's z-test is thoroughly justified in this case.

(*ii*) The variability of the production of "flowers" is altered but not the mean number of flowers. It is possible that this has caused a just appreciable effect on the variability of the proportion of "flowers : buds". In such cases the z-test would not reveal any effect.

(*iii*) In the case of "bolls" and the proportion of "bolls : buds" neither the mean values nor the variabilities appear to have altered appreciably.

8. It will be noticed from the above results that in certain cases (*e.g.*, the production of "flowers" in the cotton plant under different manurial treatments), the use of the z-test is not sufficient. It is, therefore, desirable and necessary to use the new tests of Neyman and Pearson whenever there is any suspicion of the variabilities becoming sensibly altered. The separate calculation of the variance for each sample is not difficult (in fact most of the arithmetical work is usually done in the course of the analysis of variance), and the calculation of l_0 and l_1 is also easy and straightforward and should take very little time. The expected values of l_0 and l_1, however, require very laborious calculations with Gamma functions. Tables of 5 per cent. and one per cent. values of l_0 and l_1 for a fairly large range of values of n and k have been prepared in my laboratory, and will be shortly published. With the help of these new tables, the use of the l-tests will be as easy and as simple as the use of the z-tests. It is scarcely necessary to point out that the l_0-and l_1-tests do not supplant but merely supplement the z-test.

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APPENDIX I

	Buds			Flowers			Bolls			Bud to flower			Flower to boll			Bud to boll		
	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"	"A"	Controll	"B"	"A"	Control	"B"
1	378	266	331	136	104	136	42	34	36	36·0	28·4	41·1	30·8	32·7	26·5	11·1	9·3	10·9
2	375	258	156	145	90	74	37	28	19	38·7	34·9	47·4	25·5	31·1	25·7	9·9	10·85	12·2
3	169	184	205	66	79	83	20	31	29	39·1	42·9	40·5	30·3	39·2	34·9	11·8	16·8	14·2
4	622	184	306	234	60	130	67	22	40	37·6	32·6	42·5	28·6	36·7	30·8	10·8	11·95	13·05
5	293	208	402	114	120	176	36	35	44	38·9	39·0	43·8	31·6	29·2	25·0	12·3	11·4	10·9
6	218	159	259	84	56	104	27	19	35	38·5	35·2	40·2	32·1	34·1	33·7	12·4	11·9	13·5
7	410	358	178	141	126	83	41	40	27	34·4	35·2	46·6	29·1	31·1	32·5	10·9	11·2	15·2
8	280	88	306	117	39	147	28	12	38	41·8	44·3	48·0	23·9	30·8	25·9	10·0	13·6	12·4
9	470	386	289	141	133	132	39	41	45	30·0	34·5	45·7	27·7	30·8	34·1	8·3	10·6	15·6
10	431	375	126	133	137	61	40	43	17	30·9	36·5	48·3	30·1	31·4	27·9	9·3	11·5	13·5
11	266	152	..	102	68	..	28	21	..	38·4	44·7	..	27·5	30·8	..	10·5	13·8	..
12	504	234	225	166	94	101	45	82	33	32·9	40·2	44·9	27·1	34·0	32·7	8·9	13·7	14·7
13	639	260	224	186	111	110	58	39	27	29·1	42·7	49·1	31·2	35·1	24·5	9·1	15·0	12·0
14	386	175	221	131	85	107	44	29	33	33·9	48·6	48·4	33·6	34·1	30·8	11·4	16·6	14·9
15	592	295	296	193	117	155	53	41	44	32·6	39·7	52·4	27·5	35·0	28·2	8·95	13·9	14·85
16	219	224	208	99	96	103	24	28	32	45·2	42·9	48·5	24·2	29·2	31·1	10·95	12·5	15·4
17	337	184	276	150	95	139	37	23	39	44·5	51·6	50·0	24·7	21·2	28·1	11·0	12·5	14·1
18	309	315	149	152	151	80	43	42	19	49·2	47·9	53·7	28·3	27·8	23·8	13·9	13·4	12·7
19	397	253	460	169	104	2·7	51	33	60	42·6	41·1	47·2	30·2	31·7	27·6	12·8	13·0	13·0
20	308	328	271	113	120	116	54	40	42	36·7	36·6	42·8	30·1	33·3	36·2	11·0	10·4	15·5

STATISTICAL NOTES FOR AGRICULTURAL WORKERS.

NO. 7.—THE EFFECT OF THE TIME OF APPLICATION OF FERTILIZERS ON THE YIELD AND THE RATE OF SHE DING OF BUDS, FLOWER AND BOLLS IN THE COTTON PLANT IN SURAT.

BY

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(Received for publication on 3rd October 1932)

1. Mr. K. V. Joshi of the Cotton Research Laboratory, Surat, sent us last year for statistical analysis the results (given in Appendix I) of his experiments conducted in 1930-31 with fertilizers on a selected strain of the cotton plant.

Mr. Joshi stated that the cotton plant in Surat produces on an average about 300 buds of which about 225 are ordinarily shed as buds, so that only about 75 develop into flowers. Out of these 75 flowers no less than about 45 are again shed in a premature condition, so that only about 30 (out of 75 flowers or 300 buds) mature into open bolls and thus contribute to the final yield. The effect of fertilizers is often complex. It may give increased production of buds, but if the shedding of buds, or the shedding of flowers is also increased there may not be any gain in the final yield. On the other hand even if the production of buds remains the same but the shedding of buds or of flowers is appreciably decreased, the final yield may be considerably increased. Mr. Joshi enquired how these different factors may be disentangled, and whether it is possible to construct a generalized index which would give due weight to the different factors. In our opinion the best procedure in this case would be to study each stage separately, and also to study the over-all or total yield by itself.

If we neglect any possible effect which fertilizers may have on the variability of production*, we can use Fisher's method for the analysis of the results. A discussion of Mr. Joshi's data on these lines is given below. The details of the analysis will be found in Appendix II.

2. In the first experiment with dry manures, a control plot (with 20 plants) was maintained without any artificial fertilizers, another plot (also consisting of 20 plants) was treated with 40 lbs. of nitrogen per acre applied in the form of sulphate of ammonia in the month of July, a third plot (with 19 plants) was treated with the same quantity of the same manure in August.

*The effect on variability has been considered in greater detail in a seperate note (No. 6, this Journal 3, 131.

3. The mean yields of buds per plant under the different treatments are given in Table I.

Buds.

TABLE I.

Treatment.	Mean number of buds per plant	Difference in yield from		
		Control	July-manure	August-manure
Control	254·0	..	—126·2	—3·3
July-manure	380·2	+126·2	..	+122·9
August-manure	257·3	+3.3	—122·9	..

The z-test is positive indicating a significant difference in mean values. Standard error of difference in mean=32·96.

The July application gave a definitely larger yield of buds, while the August application is practically ineffective.

4. For flowers we have the following results.

Flowers.

TABLE II.

Treatment	Number of flowers per plant	Difference in yield from		
		Control	July-manure	August-manure
Control	99·3	..	—39·3	—19·4
July-manure	138·6	+39·3	..	+19·9
August-manure	118·7	+19·4	—19·9	..

The z-test is negative, and the standard error of difference in means=22·24. Differences in the number of flowers per plant are negligible.

5. Finally for bolls the results are given below.

Bolls.

TABLE III.

Treatment	No. of bolls per plant	Difference in yield from		
		Control	July-manure	August-manure
Control	31·6	..	—8·1	—3·1
July-manure	39·7	+8·1	..	+5·0
August-manure	34·7	+3·1	—5·0	..

The z-test is on the verge of significance. Standard error of difference in means=3·20.

The July-manure produces an appreciably larger number of bolls than the untreated plants, but August-manuring is ineffective.

6. The mean percentage of success of buds developing into flowers is given in Table IV.

Flowers : Buds.

TABLE IV.

—	Mean proportion of flowers : buds	Difference from		
		Control	July-manure	August-manure
Control	39·0	..	+2·5	—7·1
July-manure	36·5	—2·5	..	—9·6
August-manure	46·1	+7·1	+9·6	..

The z-test is positive, and the standard error of difference in means=3·00.

The August-manure shows the largest percentage of flowers (that is the lowest rate of shedding of buds), the difference from the July-manured or untreated plots being statistically significant. The July-application, however, shows no advantage over the unmanured plants.

7. Mean percentage of success of flowers developing into bolls is given in Table V.

Bolls : Flowers.

TABLE V.

Treatment.	Mean proportion of bolls : flowers	Difference from		
		Control	July-manure	August-manure
Control	31·9	..	+3·3	+2·7
July-manure	28·6	—3·3	..	—0·6
August-manure	29·2	—2·7	+0·6	..

The z-test is negative, standard error of difference in means=1·55.

The application of nitrogen has no appreciable effect on the whole.

8. The mean percentage of success of buds growing into bolls is given in Table VI.

Bolls : Buds.

TABLE VI.

—	Mean proportion of bolls : buds	Difference from		
		Control	July-manure	August-manure
Control	12·4	..	+2·0	—1·1
July manure	10·4	—2·0	..	—3·1
August-manure	13·5	+1·1	+3·1	..

Standard error of difference in means=·93.

August-manuring has no effect, while July-manuring gives the worst results.

9. We may now summarize the effect of the time of application of fertilizer separately.

(*a*) *July-manuring.*

Manuring in July definitely stimulates the production of a larger number of buds per plant which is about 50 per cent. higher than that of the untreated plants. During the flowering and bolling stage, the rate of loss is slightly larger than that

of the control plot, so that ultimately the number of bolls per plant is only 26 per cent. higher than that of the untreated plants.

(*b*) *August-manuring.*

August-manuring does not lead to any appreciable increase in the number of either the buds, flowers or bolls. The rate of shedding during different stages too indicate no significant improvement over the control, except a slight stability of the buds during the flowering stage.

Summary.

Results of certain fertilizer experiments on the cotton plant conducted in 1930-31 by Mr. K. V. Joshi of the Cotton Research Laboratory, Surat, have been discussed in this paper. The effect of applying 40 lbs. of nitrogen in July to certain plants, and the same quantity of nitrogen in August to other plants has been studied in detail with reference to the following factors :- -The production of (1) Buds, (2) Flowers, and (3) Bolls, and the percentage of (4) Buds developing into flowers, (5) Flowers developing into bolls, and (6) Buds developing into bolls. It was found that manuring in July gave a significant increase in the yield of buds and flowers, and although it showed an appreciably greater rate of shedding of buds, the increase in the final yield of bolls was also found to be significant.

Fuller details of the experiment will be published by Mr. Joshi himself in due course.

REFERENCE.

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Appendix 1.

	Buds			Flowers			Bolls			Bud to flower			Flower to boll			Bud to boll		
	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"	"A"	Control	"B"
1	378	366	331	136	104	136	42	34	36	36·0	28·4	41·1	30·8	32·7	26·5	11·1	9·3	10·9
2	375	258	156	145	90	74	37	28	19	38·7	34·9	47·4	25·5	31·1	25·7	9·9	10·85	12·2
3	169	184	205	66	79	83	20	31	29	39·1	42·9	40·5	30·3	39·2	34·9	11·8	16·8	14·2
4	622	184	306	234	60	130	67	22	40	37·6	32·6	42·5	28·6	36·7	30·8	10·8	11·95	13·05
5	293	308	402	114	120	176	36	35	44	38·9	39·0	43·8	31·6	29·2	25·0	12·3	11·4	10·9
6	218	159	259	84	56	104	27	19	35	38·5	35·2	40·2	32·1	34·1	33·7	12·4	11·9	13·5
7	410	358	178	141	126	83	41	40	27	34·4	35·2	46·6	29·1	31·7	32·5	10·0	11·2	15·2
8	280	88	306	117	39	147	28	12	38	41·8	44·3	48·0	23·9	30·8	25·9	10·0	13·6	12·4
9	470	386	289	141	133	132	39	41	45	30·0	34·5	45·7	27·7	30·8	34·1	8·3	10·6	15·6
10	431	375	126	133	137	61	40	43	17	30·9	36·5	48·3	30·1	31·4	27·9	9·3	11·5	13·5
11	266	152	...	102	68	...	28	21	...	38·4	44·7	...	27·5	30·8	...	10·5	13·8	...
12	504	234	225	166	94	101	45	32	33	32·9	40·2	44·9	27·1	34·0	32·7	8·9	13·7	14·7
13	639	260	224	186	111	110	58	39	27	29·1	42·7	49·1	31·2	35·1	24·5	9·1	15·0	12·0
14	386	175	221	131	85	107	44	29	33	33·9	48·6	48·4	33·6	34·1	30·8	11·4	16·6	14·9
15	592	295	296	193	117	155	53	41	44	32·6	39·7	52·4	27·5	35·0	28·2	8·95	13·9	14·85
16	219	224	208	99	96	103	24	28	32	45·2	42·9	48·5	24·2	29·2	31·1	10·95	12·5	15·4
17	337	184	276	150	95	139	37	23	39	44·5	51·6	50·0	24·7	24·2	23·1	11·0	12·5	14·1
18	309	315	149	152	151	80	43	42	19	49·2	47·9	53·7	28·3	27·8	23·8	13·9	13·4	12·7
19	397	253	460	169	104	217	51	33	60	42·6	41·1	47·2	30·2	31·7	27·6	12·8	13·0	13·0
20	308	328	271	113	120	116	54	40	42	36·7	36·6	42·8	30·1	33·3	36·2	11·0	10·4	15·5

APPENDIX II.

Analysis of variance.

(1) Buds.

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	204,419	102,210	1·1207	·5738
Residual errors . . .	56	608,588	10,867		
Total .		813,007			

Standard error of difference in means=32·96.

(2) Flowers.

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	15,438	7,719·0	·2215	·5738
Residual errors . . .	56	276,950	4,945·5		
Total .		292,388			

Standard error of difference in means=22·24.

(3) Bolls.

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	684·69	342·35	·5718	·5738
Residual errors . . .	56	6,109·29	109·09		
Total .		6,793·98			

Standard error of difference in means=3·20.

(4) *Flowers : Buds.*

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	952·53	476·27	·8250	·5738
Residual errors . . .	56	5,032·55	91·46		
Total .		5,985·08			

Standard error of difference in means=3·00.

(5) *Bolls : Buds.*

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	108·21	54·11	·9179	·5738
Residual errors . . .	56	483·40	8·63		
Total .		591·61			

Standard error of difference in means=·93.

(6) *Bolls : Flowers.*

Variation due to	D. F.	Sum of squares	Mean square	z	5 per cent. z ($n_1=2$, $n_2=60$)
Manures	2	111·13	55·57	·4185	·5738
Residual errors . . .	56	1,347·86	24·06		
Total .		1,458·99			

Standard error of difference in means=1·55.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS.

No. 8.—THE EFFECT OF DIFFERENT DOSES OF NITROGEN ON THE RATE OF SHEDDING OF BUDS, FLOWERS AND BOLLS IN THE COTTON PLANT IN SURAT.

BY

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(Received for publication on 3rd October 1932.)

1. Mr. K. V. Joshi* of the Cotton Research Laboratory, Surat, sent us the results (reproduced in Appendix I) of certain experiments on the cotton plant conducted by him in 1930-31. The object of the experiment was to study the effect of applying different doses of nitrogen. There were altogether 3 plots each consisting of 10 plants. One plot was left unmanured to serve as control. One plot was treated with 40 lbs. of nitrogen per acre, and the other plot with 100 lbs. of nitrogen per acre applied in the form of ammonium sulphate.

If we concentrate our attention on the mean yield we may use Fisher's method of analysis of variance for disentangling the different factors. A full discussion of the results is given below. The value of z is obtained in each case by dividing the variance to be tested by the residual variance and taking half the natural logarithms. That is

$$z=\tfrac{1}{2}\log_e\left(\frac{v}{v_0}\right)$$

where v_0 is the residual variance. The critical values of (either on the basis of 5 per cent. or one per cent. probabilities) have been taken from Fisher's Table VI (Statistical Methods for Research Workers, 1930, 212-213).

* Full details of the experiments will be published by Mr. Joshi himself in due course. The present note is intended to offer a few suggestions regarding the statistical methods which would be found appropriate in such problems.

2. The analysis for the number of buds under different treatments is given in Tables I and II.

TABLE I.

(*Number of buds*).

Comparisons	—	Sum of squares	Mean square	Value of z	
				Observed	5 per cent.
Nitrogen *vs.* no nitrogen. .	1	296,995·5	296,995·5	1·0856	0·7205
Doses of nitrogen . . .	1	49,475·6	49,475·6	1·1882	0·7205
Total .	2	346,471·1			
Residual	26	876,740·7	33,875·0		
Total .	28	1,223,211·8			

TABLE II.

(*Number of buds*).

Comparisons	No. of buds	Difference from	
		Control	40 lbs. nitrogen
No manure	434·3	—	—164·5
40 lbs. nitrogen	598·8	+164·5	—
100 lbs. nitrogen	701·0	+266·7	+102·2

Standard error of difference in means=83·3.

The response due to the application of nitrogen is significant, but the differential response due to an increased dose of nitrogen is inappreciable.

3. The analysis for the number of flowers is given in Tables III and IV.

TABLE III.

(*Number of flowers*).

Comparisons	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	Expected (5 per cent.)
Nitrogen *vs.* no nitrogen .	1	76,246·2	76,246·2	1·6388	0·7205
Different doses of nitrogen .	1	21,203·2	21,203·2	0·9989	0·7205
Treatment	2	97,449·4	48,724·7		
Error	26	76,374·5	2,875·9		
Total .	28	173,823·9			

TABLE IV.

(*Number of flowers*).

Comparisons	Mean number of flowers	Difference from	
		Control	40 lbs. nitrogen
No manure	111·8	—	—76·2
40 lbs. nitrogen	188·0	+76·2	—
100 lbs. nitrogen	254·9	+143·1	+66·9

Standard error of difference in means=23·91.

Both the doses of nitrogen have significantly increased the number of flowers per plant; the additional dose of nitrogen is also effective in further stimulating the formation of flowers from buds.

4. The analysis for bolls is given in Tables V and VI.

TABLE V.

(*Number af bolls*).

Comparisons	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	5 per cent.
Nitrogen *vs.* no nitrogen. .	1	8,545	8,545	1·7130	0·7205
Different doses of nitrogen .	1	1,832	1,832	0·9428	0·7205
Treatment	2	10,377	5,189		
Error	26	7,222	278		
Total .	28	17,599			

Standard error of difference in means=7·46.

TABLE VI.

(*Number of bolls*).

Comparisons	Mean number of bolls	Difference from	
		Control	40 lbs. nitrogen
Control	36·2	—	—26·8
40 lbs. nitrogen	63·0	+26·8	—
100 lbs. nitrogen	82·6	+46·4	+19·6

Standard error of the difference in means=7·45.

The efficacy of nitrogen is definitely established. Moreover, the additional 60 lbs. of nitrogen too is found to increase the number of bolls quite appreciably.

5. We may now consider the effect of nitrogen on the rates of shedding.

The analysis of the percentage success of buds developing into flowers is given in Tables VII and VIII.

TABLE VII.

(*Flowers : Buds*).

Comparisons	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	5 per cent.
Nitrogen *vs.* no nitrogen. .	1	104·91	104·91	0·7849	0·7205
Different doses of nitrogen .	1	99·32	99·32	0·7575	0·7205
Treatment	2	204·23	102·12		
Error	26	567·88	21·83		
Total .	28	772·11			

TABLE VIII.

(*Flowers : Buds*).

Comparisons	Mean proportion of flowers : buds	Difference from	
		Control	40 lbs. nitrogen
No manure	25·7	—	—5·7
40 lbs. nitrogen	31·4	+5·7	—
100 lbs. nitrogen	36·3	+10·6	+4·9

Standard error of the difference in means=2·08.

The application of nitrogen is associated with a significant increase in the percentage success of buds growing into flowers. The additional dose of nitrogen too is effective in diminishing the rate of shedding of buds.

6. The analysis of the percentage success of flowers developing into bolls is given in Tables IX and X.

TABLE IX.

(*Bolls : Flowers*).

Comparisons	D. F.	Sum of squares	Mean variance
Nitrogen *versus* no nitrogen	1	3·24	3·24
Different doses of nitrogen	1	4·20	4·20
Treatment	2	7·44	3·72
Error	26	180·61	6·95
Total	28	188·05	

The residual mean variance is larger than that due to treatment, which at once indicates that the treatments are ineffective in diminishing the rate of shedding of flowers before the boll stage.

TABLE X.

(*Bolls : Flowers*).

Comparisons	Mean proportion of bolls : flowers	Difference from	
		Control	40 lbs. nitrogen
Control	32·3	..	—1·2
40 lbs. nitrogen	33·5	+1·2	..
100 „ „	32·5	+0·2	—1·0

Standard error of difference in means=1·20.

The differences are all statistically negligible. Thus nitrogen has no effect on the rate of shedding of flowers.

7. The analysis of the percentage success of buds developing into bolls is given in Tables XI and XII.

TABLE XI.

(*Bolls : Buds*).

Comparisons	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	5 per cent.
Nitrogen *versus* no nitrogen . . .	1	46·77	46·77	1·7544	0·7205
Different doses of nitrogen . . .	1	8·42	8·42	0·8971	0·7205
Treatment	2	55·19	27·59		
Error	26	36·32	1·40		
Total .	28	91·51			

TABLE XII.

(*Bolls : Buds*).

Comparisons	Mean proportion of bolls : buds	Difference from	
		Control	40 lbs. nitrogen
Control	8·56	..	—2·04
40 lbs. nitrogen	10·60	+2·04	..
100 „ „	11·93	+3·37	+1·33

Standard error of difference in means=0·37.

Apparently nitrogen has some effect on the percentage success of buds developing into bolls, and there is a response due to the increased dose of nitrogen.

APPENDIX.

Serial No.	Buds			Flowers			Bolls			Bud to flower			Flower to boll			Bud to boll		
	40 lbs. N_2	Control	100 lbs. N_2	40 lbs. N_2	Control	100 lbs. N_2	40 lbs. N_2	Control	100 lbs. N_2	40 lbs. N_2	Control	100 lbs. N_2	40 lbs. N_2	Control	100 lbs. N_2	40 lbs. N_2	Control	100 lbs. N_2
1	551	426	651	148	100	228	58	32	72	26·8	23·4	35·0	39·2	32·0	31·5	10·5	7·5	11·0
2	749	439	459	220	119	183	78	43	57	29·4	27·1	39·8	35·5	36·1	31·1	10·4	9·8	12·4
3	413	512	838	145	133	334	49	51	109	35·1	26·0	39·8	33·8	38·3	32·6	11·8	10·0	13·0
4	616	302	551	213	102	220	77	31	79	34·6	33·7	39·9	36·2	33·3	35·9	12·5	10·2	14·3
5	614	324	730	179	88	269	60	27	85	29·1	27·1	36·8	33·5	30·6	31·6	9·7	8·3	11·6
6	989	248	1,100	292	88	380	94	25	115	29·4	35·5	34·5	32·2	28·4	30·2	9·5	10·1	10·4
7	392	636	..	127	146	..	42	46	..	32·4	22·9	..	33·1	31·5	..	10·7	7·2	..
8	790	407	839	267	99	293	82	33	92	33·8	24·3	34·9	30·7	33·3	31·3	10·4	8·3	10·9
9	452	349	463	151	83	162	49	26	58	33·4	23·7	35·0	32·4	31·3	35·8	10·8	7·4	12·5
10	422	700	678	138	160	225	41	48	77	32·7	22·8	33·1	29·7	30·0	34·2	9·7	6·8	11·3

ABSTRACT

A new method of determining clay content of soils by moisture absorption at 70 per cent. humidity.* AMAR NATH PURI. *Soil Science* **33**, No. 6, June, 1932.

Moisture from the vapour phase may be absorbed by soils in three ways. It may enter into loose chemical combination and exist as water of hydration, it may be taken up in the minute capillaries on the surface of individual particles, or it may fill up the interstitial spaces between the aggregates of various particles. The vapour pressure curve for soils could be split up into three portions, the portion of the vapour pressure curve below 10 per cent. humidity representing water of hydration, that between 10-70 per cent. humidity capillary absorbed water and that above 70 per cent. interstitial water. Water of hydration will depend on the constitutional nature of soil colloids, the capillary absorbed water on the extent of the surface area and interstitial water on the state of aggregation. It is claimed that the correctness of this hypothesis in the cases of capillary absorbed water is shown by the fact that this moisture is correlated with the total clay content. In the case of interstitial water it is supported by the fact that there is an independent method of determining the state of aggregation of the soil particles, *i. e.*, the measurement of the Dispersion Coefficient, and it is seen that soils in general show a positive correlation between interstitial water per unit surface and Dispersion Coefficient. The correlation coefficient for 57 soils examined in this investigation, worked out to be 0·765, which is highly significant.

A sharp distinction between the three forms of hygroscopic moisture is not possible by the very nature of things. For one thing there are no sharp breaks in the vapour pressure curve; but from an examination of these curves it may be safely stated that fixing 10-70 per cent. humidity limits, will eventually help in elucidating the mechanism of moisture absorption much better than the simpler conception of the hygroscopic moisture as a uniform film of water round the soil particles.

It is claimed that the clay (0·001 m. m.) content of a soil can be estimated from moisture absorption between 10-70 per cent. humidity with a fair amount of accuracy, by the help of the following empirical formulae. (i). Clay $= 8{\cdot}04\ H + 1{\cdot}02$ and (ii) Clay $= 8{\cdot}41\ H' + 2{\cdot}8$ where H and H′ are hygroscopicity values when the equilibrium is reached by drying and wetting respectively.

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ORIGINAL ARTICLES

THE INTAKE OF NITROGEN BY THE RICE PLANT (*ORYZA SATIVA* L.)

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The intake of nitrogen by the rice plant has been a subject of investigation for the last fifty years. Kellner [1884] was perhaps the first to conduct water-culture experiments on lowland rice to determine the form of nitrogen in which the rice plant thrives best. He found that the rice plant needed ammoniacal nitrogen during the early stages and nitrate nitrogen in the later stages of growth. Nagaoka [1904] also, as a result of a large series of plot and pot-culture experiments, showed that in ammonium sulphate the rice plant made better growth than in the nitrate salts. Nagaoka [1903, 1906] attributed the inability of the rice plant to make use of nitrate nitrogen to the deficiency of sugar in leaves to convert nitrates into proteins. The conclusion of Kellner [1884] and Nagaoka [1904] were confirmed by Daikuhara [1905] who also found ammonium sulphate a better fertilizer than sodium nitrate. Similar results were also obtained by Aso and Bahadur [1906], by

Krauss [1907] and by Trelease and Paulino [1920]. Kelley [1911] also established the superiority of ammonium sulphate as a fertilizer over other nitrate salts by means of field and pot-culture experiments. He also showed that the application of ammonium sulphate before planting resulted in better growth than when applied at short intervals during the culture period. while manuring with nitrate at flowering stage proved more beneficial than early manuring with the same salt. These findings were further supported by later work of Kelley [1914] who conducted field experiments. Daikuhara and Imrseki [1907] also found that ammonium sulphate was better than sodium nitrate or a combination of both.

W. H. Harrison [1912] also found that ammonium salts which yield ammonia under anaerobic conditions were suitable for the rice plant, while J. B. Harrison [1913] found that the ammonium sulphate brought about a decrease in yield.

Espino [1920] studied the salt requirements of the young rice plant by means of a large series of pot-culture experiments and he used three different types of solutions. As the effect of ammonium ion had to be studied, he could not use 3-salt solution of Shive [1915], but had to use a four-salt type of solution as other three kations are equally important. He found the best growth in a solution containing monopotassium phosphate, calcium nitrate, ammonium sulphate and magnesium sulphate of about ·002 *M* per litre with an osmotic concentration of about ·08 atmosphere. The results indicate that the rice plants require both ammonium and nitrate ions.

Trelease [1920] in his study on the growth of rice in relation to the different proportions of fertilizer salts found that ammonium sulphate brought about a marked improvement in the growth of the rice plant while calcium phosphate or potassium sulphate had no influence upon the plants.

Numerous explanations have been proposed to explain the fact that some plants like rice respond better to ammoniacal nitrogen than to nitrate nitrogen. Willis and Carrers [1923] suggest that superiority of ammonium ion over nitrates for the rice plants is based on the toxic influence on the plants of the basic residues of nitrate salts. Calcium nitrate is as suitable as ammonium sulphate if the unassimilated residue of calcium nitrate does not interfere with the absorption of iron. Kinoshita [1894-1897] on the other hand thinks that ammonium salts are rapidly converted into asparagin while nitrates tend to accumulate and fail to increase the amount of asparagin.

It is also determined by water-culture or pot-culture experiments which concentration of the different salts is most beneficial to the growth of the rice plant.

In all the work done on the salt requirements of the rice plant the absorption of the essential elements by the rice plant is determined by finding out the chemical composition of the plant at different stages of growth, and the importance of a

particular element in the nutrition of the plant is determined by the resultant growth or by the yield of grain when that particular element is added to the culture solution. Gericke [1930] has recently shown that the actual salt requirement of the rice cannot be determined by growing plants to maturity in so-called complete nutrient solutions. His results have shown that the amounts of salts absorbed, if they are available continually, are generally larger than are required for the growth shown by the plant. If the salt of a certain concentration is supplied to the plant during a certain period in the growth cycle, it is found adequate and a further supply is unnecessary.

The above investigations, though so very important from the agricultural point of view, do not afford quantitative data as to the intake of salt. The quantitative estimations of the salts taken up by the rice plant from the known concentrations of external solutions are necessary for an analysis of the complex and little-understood phenomenon of the intake of salts and to formulate general laws showing the relations of electrolytes to living tissues.

In the earlier work on the entry of solutes in plant cells it was assumed that the plants absorbed salts as such, but the recent investigations have shown that the above assumption is erroneous, at least in some cases, and the two ions of a salt are absorbed unequally. Meurer [1909] made direct observations on the intake of different ions of a salt by plant tissues like that of carrots and beet roots and showed that with carrot the kations of chlorides are absorbed to a greater extent than the chlorine ions, while with the beet the differences in the absorption of the two ions are still greater. The same author showed that the unequal absorption took place in the living tissues and not in the dead tissues. Pantanelli and Sella [1909] studied the unequal absorption of ions by *Cucurbita pepo* from culture solutions of different concentrations and the results show that anion was absorbed in excess of the kation. Pantanelli [1915] then extended his observations to a number of other species and found that the unequal absorption of ions was of a general occurrence. The unequal absorption of ions from a solution of calcium chloride by *Pisum sativum* and *Zea Mays* was shown by Redfern [1922] and she found that the unequality of absorption of the two ions decreased as the concentration of the solution was decreased.

The unequal absorption of the two ions from a salt solution leads to other changes in external solution. The absorption of an excess of one ion into the plant cannot take place without replacement of the excess with an equal quantity of another ion carrying the same charge. If the chloride ion is absorbed in excess of potassium ion from a solution of potassium chloride, an equal quantity of other ion carrying the same charge as the chloride ion must take its place. Either the hydroxyl ion derived from the solvent appears to replace the excess of chloride

ion absorbed or an equivalent quantity of some negatively charged ion diffuses out from the plant. If the former is the case it is assumed that the liberated hydrogen-ion of the solvent is absorbed by the plant along with the excess of chloride ion. Thus the unequal absorption of an ion of a salt would render the solution alkaline in this particular case. The solution would become acidic if the potassium ion absorbed is in excess of the chloride ion as the hydrogen ion liberated from the solvent will replace the excess of the kations absorbed. In the second case the external solution will remain the same as the excess of an ion absorbed is replaced by exosmosis of ions carrying the same charge. The latter was the case in the experiments performed by Redfern [1922] when the diffusion of potassium and magnesium took place from the roots.

It was also formerly believed that the passage of dissolved substances in solution into plant cells continued to take place until the concentration outside and inside the plant became equal. But Pfeffer [1900, 1903] showed that the concentration of a substance inside a cell remained greater or less than its concentration outside the cell. The position of equilibrium attained in the passage of dissolved salts may vary with different substances and in different species. In order to determine the point of equilibrium it is necessary to determine the final internal concentration and the final external concentration of a salt when further intake of the salts ceases. This is termed absorption ratio by Stiles and Kidd [1919]. The absorption ratios for a number of salts and dyes in solution of different strengths are determined by Stiles and Kidd [1919] and other workers. They found that a plant cannot absorb chlorides of potassium, sodium and calcium in all concentrations and the absorption is in the beginning proportional to the concentration of the solution outside but later the same relations between the internal and external concentrations are not maintained. With low concentration the absorption ratio is more than unity, but with increasing concentration it diminishes.

The relation between the final internal concentration y and the final external concentration c is given by the equation $y = k\,c^m$ where k and m are constants.

The position of equilibrium is also dependent upon the nature of the absorbed salt. Basic dyes are absorbed to a greater extent than the acid dyes. If the acid radical is the same, the salts are absorbed in the order $K^{\cdot}$, $Na^{\cdot}$, $Li^{\cdot}$, ($Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$), $Al^{\cdot\cdot\cdot}$, while if the basic radical is the same the salts are absorbed in the order NO_3', Cl', SO_4''.

The absorption of a salt is also greatly influenced by the presence or absence of another salt in the external solution. It was shown by Brenchley [1910, 1914], Stiles and Jorgensen [1914] that a second substance dissolved in a solution reduces the harmful effect of another substance and this is probably due to the hindrance

of the substance against another. Such cases of antagonism between monovalent and divalent kations are well-known and a large amount of literature has been produced on the subject by the work of Loeb [1897, 1900, 1901, 1902, 1905, 1906], Osterhout [1906], Brenner [1920] and others.

The intake of salts by the rice plant has not been studied from the above-mentioned points of view and the study of the intake of salts like ammonium salts and nitrates would prove of great value. The determination of absorption ratio for the essential salts in different concentrations of the salt would prove important as it would be possible to determine the best concentration of the salt which would be both adequate and economical. It would also be interesting to study the absorption ratio of a salt when supplied singly and when supplied in presence of other salts and whether the presence of other salts alters its absorption ratio or not.

The experimental work on the intake of salts can be done in two ways. In one case isolated cells or tissues of known volume are taken for experiment. The tissues are kept in a known volume of a solution of known concentration and the change in the electrical conductivity of the external liquid is measured after immersion. In this way the change in ionic concentration of the external solution could be known. In other cases the change in the external solution is measured by direct chemical analysis. In the second method the whole living plant is kept in a water-culture solution. The second method is generally used for determining the relationship between the composition of the culture solution and the growth of the plant, and is not used for obtaining data for the intake of salts as is proposed to do in this investigation.

For the purpose of the investigation the first method is not suitable as it is undertaken to determine the intake of salts by the whole living plant as isolated strips of tissues of the roots would not yield reliable results. Secondly the external solution is analysed by chemical methods and not by a change in the electrical conductivity of external liquids as the latter would not give any information regarding the relative absorption of the two ions of a salt dissolved in the solution.

Investigation.

The rice plants used in this investigation are either grown in culture solution from the very beginning of the germination of the seeds or taken from the experimental beds at different stages and kept in culture solutions. For the earlier stages of growth generally the rice plants grown in culture solutions from the beginning of germination are used, while for the advanced stages the plants from the beds are also used. The plants removed from the beds grow healthy and normally in culture solution and do not suffer from the transference from the soil to glass jars contain-

ing the culture solution. It was also possible to grow plants in culture solution from the germination stage upto the flowering stage but the vigour of the plant is not the same as those grown in the soil. For successful water culture experiments it is necessary to put the seedlings in culture solution on the fifth or sixth day after germination in saw-dust.

It is also necessary to keep the culture jars buried in saw-dust or soil in a wooden box so that only the mouths of the culture jars project above the surface. It is found in practice that the growth of the seedlings in culture jars thus embedded in soil or saw-dust is better than if they are not embedded but made only dark by covering with black paint or paper. Embedding serves two purposes, firstly it keeps the temperature of the solution low at mid-days and secondly it completely cuts off the light from reaching the roots.

In the beginning the culture experiments proved a failure as the plants would not grow. All the unsuccessful attempts are not described here. It was after a great deal of experience that the plants could be grown by the water-culture method.

It was first undertaken to study the intake of ammonium sulphate by the germinating seeds as it is now known that a dose of ammonium sulphate in the seedling stage is found helpful for healthy growth. Though ammonium sulphate is known for its value as a fertilizer, no quantitative data exist about its intake by the plants in the early stages. It would be of interest to determine the amount of the two ions taken up by the seedlings from a known concentration of the solution. Seeds were sown in saw-dust and on the ninth day after sowing thirty-six seedlings apparently of the same size and vigour of growth were placed in ·002 *N* ammonium sulphate solution. Ten such sets of experiments were started on the same day. Sets were kept buried in moist sand, in order to keep the temperature low, and also to prevent sunlight reaching the roots. All the precautions necessary for the culture experiments were taken specially against the attacks of fungi and from the pressure of cotton wool on the side of seedlings which was used for their support. The original ·002 *N* ammonium sulphate solution was estimated for SO_4'' and $NH_4^{\cdot}$ ions. SO_4'' was estimated as barium sulphate by gravimetric method given by Cumming and Kay [1928] in Quantitative Chemical Analysis. Necessary precautions were taken as recommended by Cumming and Kay [1928]. Precautions as recommended by Sutton [1876] and by Treadwell and Hall [1913] and by Skinner, and others [1930] were taken for estimating ammonia. The ammonium sulphate was recrystallised several times before use. The ammonium sulphate solution was estimated for $NH_4^{\cdot}$ and SO_4'' ions every twenty-four hours after the plants were placed in the culture jars so that the quantities of the two ions absorbed by the plants after they had remained in the solution for a period of one day to ten days

could be known. 1000 c.c. of 0·002 *N* ammonium sulphate solution was kept in each culture vessel. The results are given below :—

TABLE I.

Daily absorption of NH_4· and SO_4'' ions by the rice seedlings from 0·002 N $(NH_4)_2$ SO_4 *for ten days.*

Age of the seedlings in days	Number of days in solution	Absorbed amount in grms.		Percentage of absorbed amount	
		SO_4''	NH_4·	SO_4''	NH_4·
8-9	1	0·0029	0·0018	3·0	5·0
8-10	2	0·0040	0·0029	4·2	8·0
8-11	3	0·0067	0·0047	7·0	13·0
8-12	4	0·0081	0·0058	8·4	16·0
8-13	5	0·0106	0·0072	11·0	20·0
8-14	6	0·0119	0·0083	12·4	23·0
8-15	7	0·0139	0·0097	14·5	27·0
8-16	8	0·0154	0·0112	16·0	31·0
8-17	9	0·0159	0·0117	11·5	32·5
8-18	10	0·0159	0·0118	16·5	32·5

The results show many features of interest. The NH_4· and SO_4'' ions are absorbed in unequal amounts. The former is absorbed to a greater extent than the latter as the ratio by weight of SO_4'' to NH_4· in ammonium sulphate molecule is 2·6:1. This unequal absorption of the ions is maintained for all the ten days the

plants are kept in solution. After eight days the quantities of the two ions absorbed do not increase showing that the absorption of the two ions completely stops after nine days.

The fall in the absorption of the two ions of the salts is accompanied by a fall in the absorption of water as the following measurements of the volume of culture solution in the 10 jars before estimation were made. 1000 c.c. of solution were used in all the ten culture jars.

TABLE II.

The amounts of water absorbed by the rice seedlings from 0·002 N $(NH_4)_2 SO_4$.

No. of days in solution	Initial volume taken	Volume of the remaining $(NH_4)_2 SO_4$ solution	The amount of water absorbed
	c.c.	c.c.	c.c.
1	1000	985	15
2	1000	976	24
3	1000	961	39
4	1000	952	48
5	1000	940	60
6	1000	931	69
7	1000	918	82
8	1000	908	92
9	1000	904	96
10	1000	900	100

The absorption of water nearly stops after the absorption of ion ceases. This point becomes clear if the daily quantities of the absorbed ions and water are obtained by subtracting the upper volumes from the ones next below in Tables I and II.

TABLE III.

Relation between the absorption of NH_4· and SO_4″ ions and the amount of water absorbed by the rice seedlings from 0·002 N $(NH_4)_2 SO_4$.

Age of the seedlings in days	Percentage absorption of		Absorption of water in c.c.
	SO_4″	NH_4·	
8-9	3	5	15
9-10	1·2	3	9
10-11	2·8	5	15
11-12	1·4	3	9
12-13	2·6	5	12
13-14	1·4	3	9
14-15	2·1	4	13
15-16	1·5	4	10
16-17	0·5	1·5	4
17-18	0	0	4

The figures indicate that the quantity of water absorbed is clearly related to the quantities of ions absorbed on each day. 12 to 15 c.c. of water are absorbed

when 2·8 per cent. of SO_4'' and 5 per cent. of the NH_4' ions are absorbed. The quantity of water absorbed is 4 c.c. when the absorption of the ion ceases altogether.

In a second series of experiments the rice seedlings after germination were kept in ammonium sulphate of different concentrations varying from 0·005*N* to 0·0011*N* and each solution was analysed for the NH_4' and SO_4'' ions after eight days. The results obtained would give the relative absorption of the two ions in different concentrations of the external solution.

TABLE IV.

The absorption of NH_4' and SO_4'' from $(NH_4)_2\,SO_4$ solutions of the different concentrations by the rice seedlings.

* Strength of $(NH_4)_2\,SO_4$	Absorption of SO_4'' in grams	Absorption of NH_4' in grams	Percentage absorption of SO_4''	Percentage absorption of NH_4'	Ratio of SO_4'' : NH_4' absorbed
$\frac{N}{200}$	0·0503	0·0190	20·9	21·1	2·60 : 1
$\frac{N}{300}$	0·0446	0·0180	27·8	29·9	2·40 : 1
$\frac{N}{400}$	0·0244	0·0138	20·3	30·6	1·70 : 1
$\frac{N}{500}$	0·0160	0·0116	16·7	32·2	1·30 : 1
$\frac{N}{600}$	0·0115	0·0098	14·3	32·0	1·10 : 1
$\frac{N}{700}$	0·0092	0·0088	13·4	34·2	1·00 : 1
$\frac{N}{800}$	0·0055	0·0085	9·1	37·8	0·64 : 1
$\frac{N}{900}$	0·0045	0·0084	8·4	42·0	0·53 : 1

* The strength of the different solutions used in this investigation are given in the tables in the fractions of normality and not in decimals for the sake of clarity as the differences in strengths if stated in decimals will be very small and not be correctly indicated.

The results show that the amounts of sulphate and ammonium ions absorbed by the seedlings decrease as the concentration of ammonium sulphate solution is decreased. But if the results are calculated as percentages of the total quantities of $NH_4^{\cdot}$ and $SO_4^{''}$ ions present in each solution of ammonium sulphate, it is seen that the percentage of $SO_4^{''}$ ions absorbed decreases and the percentage of $NH_4^{\cdot}$ ions absorbed increases as the concentration of the ammonium sulphate decreases. It is therefore evident that the two ions, $NH_4^{\cdot}$ and $SO_4^{''}$, are not absorbed in the same proportion as they are present in the molecule of ammonium sulphate. The results showing the proportion of the $NH_4^{\cdot}$ ion to the $SO_4^{''}$ ion absorbed from each solution of ammonium sulphate are also given above.

There is unequal absorption of $SO_4^{''}$ and $NH_4^{\cdot}$ ions from the solution of ammonium sulphate of lower concentration than $0{\cdot}005N$. There is equal absorption of the two ions in $0{\cdot}005N$ ammonium sulphate solution and then the absorption of $SO_4^{''}$ is less in proportion to the $NH_4^{\cdot}$ ion absorbed.

It may also be mentioned that the seedlings apparently did not thrive in the solution of lower concentrations than $0{\cdot}0025N$ as well as in other solutions. In the above set of experiments with solutions of different concentrations, the seedlings are kept for eight days. The period of eight days for keeping plants in each solution was chosen as estimations of the ions absorbed from $0{\cdot}02N$ solution of ammonium sulphate by the seedlings showed that after eight days the absorption of the ion was practically very little, and the point of equilibrium was reached between external solution and the internal concentration of the salt and the further intake of salts had stopped. So henceforth in all the experiments mentioned below the plants or the seedlings were kept for eight days in each solution after which the external solution was analysed.

It would also be possible from the results obtained to calculate the absorption ratio for the two ions of ammonium sulphate. The final external concentration of the solution could be determined by the amount of each ion present in the external solution after eight days and the final volume of the solution. The internal concentration of the ions could be calculated from the amount of ions absorbed and the volume of the seedlings. The total volume of the seedlings was determined in each set of experiments. The volume varied from 25 c.c. to 28 c.c. The amount of the salt in the same volume of the plant body as the volume of external solution is calculated in order to compare the two. The external and internal concentrations are given in the following tables as the quantities of ions present in equal

volumes and the absorption ratio are calculated by dividing the final internal concentration (y) by the final external concentration (c).

TABLE V.

Absorption ratios for SO_4'' ions from different concentrations of $(NH_4)_2\ SO_4$ for the rice seedlings (8-15 days old).

Strength of the $(NH_4)_2\ SO_4$ solution	Final external concentration of SO_4'' solution	Final internal concentration of SO_4''	Absorption ratio $\frac{y}{c}$
$\frac{N}{200}$	0·1897	1·6590	8·7
$\frac{N}{300}$	0·1155	1·4270	12·3
$\frac{N}{400}$	0·0957	0·8050	8·4
$\frac{N}{500}$	0·0801	0·5610	7·0
$\frac{N}{600}$	0·0686	0·4210	6·4
$\frac{N}{700}$	0·0594	0·3220	5·9
$\frac{N}{800}$	0·0546	0·1925	3·5
$\frac{N}{900}$	0·0488	0·1575	3·2

TABLE VI.

Absorption ratios for NH_4· ions from different concentrations of $(NH_4)_2\,SO_4$ for the rice seedlings (8-15 days).

Strength of the $(NH_4)_2\,SO_4$	Final external concentration of NH_4·	Final internal concentration of NH_4·	Absorption ratio $\frac{y}{c}$
$\frac{N}{200}$	0·0714	0·627	9·7
$\frac{N}{300}$	0·0422	0·576	13·6
$\frac{N}{400}$	0·0314	0·455	14·4
$\frac{N}{500}$	0·0245	0·406	16·5
$\frac{N}{600}$	0·0210	0·343	16·3
$\frac{N}{700}$	0·0170	0·308	17·5
$\frac{N}{800}$	0·0141	0·297	21·0
$\frac{N}{900}$	0·0117	0·294	25·9

The absorption ratios for the SO_4'' ions are all different for the different concentrations of the ammonium sulphate solution and the position of equilibrium attained, when no further intake of the ion takes place, varies according to the concentration of the solution. The greater absorption ratio for the sulphate ion is obtained in 0·0033*N* ammonium sulphate solution. At higher or lower concentrations of the solution of ammonium sulphate than 0·0033*N* the values of the absorption ratios for the SO_4'' ions fall.

The case is different for the absorption of NH_4· ions. The values of the absorption ratio continue to increase as the concentration of the ammonium sulphate decreases.

Proportionately more and more of the NH_4· ions are absorbed as the concentration of the solution is decreased. The absorption ratios for the NH_4· and SO_4''

ions were also calculated in the same manner for each period of 24 hours after the plants were placed in 0·002*N* ammonium sulphate solution for 24 hours to note the daily changes in values of the absorption ratios. The results are given below.

TABLE VII.

*Daily change in the absorption ratio of SO_4'' ions in 0·002*N $(NH_4)_2\ SO_4$ solution.

Time of immersion in culture, in hours	Final external concentration of SO_4'' ions (c)	Final internal concentration of SO_4'' ion (y)	Absorption ratio $\frac{y}{c}$
24	0·0932	0·0928	0·99
48	0·0921	0·1300	1·40
72	0·0894	0·2140	2·40
96	0·0880	0·2590	2·90
120	0·0855	0·3290	3·90
144	0·0842	0·3680	4·40
168	0·0822	0·4300	5·10
192	0·0807	0·4620	5·70
216	0·0802	0·4770	5·90
240	0·0802	0·4770	5·90

TABLE VIII.

Daily changes in the absorption ratios of NH_4· for rice seedlings in 0·002N $(NH_4)_2SO_4$ solution.

Time of immersion in culture	Final external concentration for NH_4· ion (c)	Final internal concentration for NH_4· ion (y)	Absorption ratio $\frac{y}{c}$
Hours			
24	0·0342	0·0576	1·7
48	0·0331	0·0942	2·8
72	0·3130	0·1500	4·8
96	0·3020	0·1860	6·1
120	0·2860	0·2230	7·7
144	0·2770	0·2570	9·2
168	0·2630	0·2950	11·2
192	0·2480	0·3360	13·5
216	0·2430	0·3510	14·4
240	0·2430	0·3510	14·4

The results show that there is no change in the absorption ratios of the two ions after eight days. It clearly shows that the points of equilibrium are finally reached on the 9th day.

It was then undertaken to determine the absorption of nitrogen or nitrate ions by the plants. Seedlings of eight days old were kept in different strengths of potassium nitrate solution ranging from 0·01*N* to 0·001*N*, but they all withered away showing that pure solutions of the nitrate in these concentrations were not

suitable for the normal growth. Only 0·00 066*N* to 0·0005*N* solution of potassium nitrate proved non-injurious to the plants and estimations of the absorption of the nitrate were made.

TABLE IX.

Quantity of NO_3' ion absorbed by the rice seedlings from KNO_3 solutions.

Strength of the KNO_3 solution	Amount of the NO_3' ion absorbed	Percentage of the NO_3' absorbed
0·00066*N*	0·0018 grm.	4·3
0·0005*N*	0·0017 grm.	5·48

The quantity of NO_3' ion absorbed is comparatively very little. NO_3' was estimated according to the method given by Cumming and Kay [1928]. All the necessary precautions were taken as recommended by them.

Experiments were then tried to see the course of absorption of $NH_4^{\cdot}$, SO_4'', NO_3' ions from a solution containing ammonium sulphate and potassium nitrate. This would enable us to determine if the presence of NO_3' ions in any way influences the absorption of $NH_4^{\cdot}$ and SO_4'' ions and it would be also of interest to determine the position of equilibrium for the different ions and how the absorption ratios for one ion is influenced by the presence of other ions. Table X below gives the results of a set of experiments with seedlings of the same age as before.

TABLE X.

Quantities of NO_3', SO_4'' and $NH_4^{\cdot}$ ions absorbed from a mixture of $(NH_4)_2SO_4$ and KNO_3 solution.

Strength of the solution in culture	Percentage of $NH_4^{\cdot}$ absorbed	Percentage of SO_4'' absorbed	Percentage of NO_3' absorbed
0·0033*N* $(NH_4)_2SO_4$ . .	28	29	..
0·00166*N* $(NH_4)_2SO_4$ . .	28	28·9	5·37
0·0005*N* KNO_3 . . .			

The results in the above table as well as the results obtained with a set of other experiments to be described below show that the absorption of the $NH_4^{\cdot}$, SO_4'' and NO_3' ions is independent of each other. The presence of nitrates does not interfere with the absorption of $NH_4^{\cdot}$ and SO_4'' ions. It is also noticed that nitrates in any concentration in presence of other salts are not injurious to rice seedlings as was found when used alone.

Experiments were done with culture solutions containing the $NH_4^{\cdot}$, SO_4'' and NO_3' ions, to see if the absorption of the three ions is independent of the presence

of foreign ions. These determinations are important as the further investigation of the intake of $NH_4^{\cdot}$ and NO_3' ions during the life-time of the plant would greatly depend upon it. If the ions $NH_4^{\cdot}$, NO_3' are absorbed independently of the presence of others it would then be possible to determine their absorption at frequent intervals in the growth cycle of the plant by keeping them in culture solutions containing all the essential elements which are required for its normal growth. It is not possible to grow plants in healthy condition in solution of a single salt and it has been found to hold good for the rice plant. Three sets of culture solutions were prepared. The solutions were 4-salt solutions first used by Tottingham [1914] as it was not possible to use the 3-salt solution of Shive [1915] on account of the inclusion of ammonium ions. One culture solution in addition to other essential elements contained nitrogen in the form of nitrate. The second solution similarly contained nitrogen in the form of ammonium sulphate and the third solution contained nitrogen both in the form of nitrate and ammonium sulphate. The 1st and 2nd solutions contained :—

$0{\cdot}001N$ KNO_3 or $0{\cdot}0015N$ $(NH_4)_2\,SO_4$.

$0{\cdot}0004N$ $MgSO_4$, $0{\cdot}00058N$ $CaSO_4$.

$0{\cdot}0011N$ KH_2PO_4 and 5 c.c. of 0·5 per cent. $FeCl_3$.

The third culture solution contained, in addition to the three salts, $\cdot 0005N$ potassium nitrate and $\cdot 00075N$ ammonium sulphate.

The strength of potassium nitrate and ammonium sulphate halved in the culture solution No. 3. The osmotic values of the solutions are given below :—

I. Osmotic pressure of solution No. (1)=0·0376 atm.

II. ,, ,, ,, ,, ,, (2)=0·0323 ,,

III. ,, ,, ,, ,, ,, (3)=0·0350 ,,

The rice plants used for the following experiments were transplanted seedlings removed from the soil and the rice seedlings grown in the normal culture solution from the very beginning of the germination of seedlings. A rice plant removed from the soil remained fresh and healthy till the end, and did not show signs of injury. The rice seedlings for these experiments were obtained from the Rice Research Station at Karjat and transplanted on the 3rd July 1929 in the beds in the garden of the Institute. Ten plants were removed from the bed and kept in 10 jars containing different culture solutions on July 8, 1929. The plants were taken out and kept in distilled water after every eight days for 24 hours. The volume of the plants was noted in each case. The quantities of $NH_4^{\cdot}$, SO_4'' and NO_3' ions absorbed after a plant had remained in the culture solution for eight days were determined. The same plant was transferred to distilled water for one day and then kept again in a fresh culture solution of the same type. This process was repeated eight times at regular intervals from 8th July to 30th October 1929.

In the case of the rice seedlings grown from the very beginning in the culture solution the same procedure as described above was adopted. The number of rice seedlings in each jar was twelve. The number was specially selected to make the volume of the twelve seedlings nearly equal to the volume of the one rice seedling taken from the bed as the size of the seedlings from the bed is much larger than the size of the seedling grown in the culture solution from the beginning of the germination of the seed. This second set of experiment is, though not quite necessary as the experiments in the 1st set were done in triplicate, is done to see how the seedlings grown in culture solution from the beginning behave in comparison to the rice seedlings grown in the soil.

In Table XI are given the quantities of $NH_4^{\cdot}$, SO_4'' and NO_3' ions absorbed during the season by the rice plants transferred to the three culture solutions from the bed. The results are expressed in percentages of the ions present in the solutions. In all the results given below, the term 'rice plant' refers to the rice plants transferred to the culture solution in July from the soil and 'rice seedlings' refers to the rice plants grown in culture solution from the beginning of the germination.

TABLE XI.

Percentage absorption of $NH_4^{\cdot}$, SO_4'' and NO_3' ions by the rice plant from the three culture solutions at different stages of growth.

Date	Culture solution No. 1		Culture solution No. 2		Culture solution No. 3		
	NO_3'	SO_4''	$NH_4^{\cdot}$	SO_4'	NO_3'	NH_4''	$SO_4^{\cdot}$
1929							
21st July to 29th July . .	8·2	17	24·4	17·1	9·7	24·8	16·9
10th August to 18th August .	10·9	13	20	12·9	11·2	19·7	12·9
21st August to 29th August .	14·8	11·9	14·9	11·9	16·1	15·3	11·9
10th September to 18th September	19·7	9	9·8	8·9	19·4	10·2	9
21st September to 29th September	22·9	9·9	13·9	10	22·3	13·2	10
11th October to 19th October .	29·5	9·9	11·7	10	29·3	11·7	10
21st October to 29th October .	19·6	7·8	9·1	7·6	19·3	9·1	7·7

The study of the figures obtained for the absorption of the $NH_4^{\cdot}$, SO_4'' and NO_3' ions from the three different culture solutions brings to light some facts not known before. If the figures are read in the horizontal line for each age of the plant, the percentage absorption of each of the three ions is practically the same in the three solutions. The $NH_4^{\cdot}$ and SO_4'' ions are absorbed in equal amounts from all the three solutions.

The same is the case for the percentage of the NO_3' ions absorbed from the culture solutions 1 and 3. The nitrate ion is absorbed in the least quantity in the early stages. The presence of nitrogen in the form of $NH_4^{\cdot}$ ion does not make any difference with the absorption of the nitrogen in the form of nitrate.

The independent entry of the three ions is maintained for all the stages of growth of the rice plant as could be seen from the results.

The study of the figures in the vertical line indicate that the percentage absorption of the $NH_4^{\cdot}$ and SO_4'' ions from the same three culture solutions begins to fall as the plant ages. For $NH_4^{\cdot}$ ions the percentage absorption falls from 28 to 9·1. There is a slight increase in the absorption in September-October very probably during the reproductive period *i.e.*, when the plant is flowering. Similarly there is a slight increase in the absorption of the SO_4'' ions during the same period. At the end of October the absorption of the SO_4'' falls to 7·8 per cent.

With the NO_3' ions the case is the reverse of the $NH_4^{\cdot}$ and SO_4'' ions. Here the percentage absorption increases as the plant ages and reaches its maximum of 29·5 per cent. in the middle of October. This clearly shows that the nitrogen in the form of nitrates is more and more absorbed as the growth of the rice plant proceeds and at the flowering stage the maximum is reached. Then there is a big fall in the percentage absorption of the nitrate ions though it is still being taken in a good quantity.

The results obtained for the seedlings grown in the culture solution from the beginning bring out the same facts about the relative absorption of the three ions.

TABLE XII.

Percentage absorption of $NH_4^{\cdot}$, SO_4'' and NO_3' ions by the rice seedlings from the 3 culture solutions at different stages of growth.

Date	Culture solution No. 1		Culture solution No. 2		Culture solution No. 3		
	NO_3'	SO_4''	$NH_4^{\cdot}$	SO_4''	NO_3'	$NH_4^{\cdot}$	SO_4''
8th to 16th July . . .	4·5	28·9	28·0	28·4	5·0	28	27·8
18th to 26th July . . .	8·0	17·0	23·9	16·9	8·0	23·9	17·2
8th to 16th August . . .	9·8	13·0	20·0	12·9	10·8	20·0	13·0
19th to 27th August . . .	14·1	12·0	15·2	10·1	15·3	15·7	12·0
8th to 16th September . .	18·5	9·0	10·0	8·9	19·6	10·2	9·0
19th to 27th September . .	22·8	9·7	14·1	9·9	22·8	14·1	9·9
9th to 17th October . . .	29·3	10	12·8	10	28·2	12·6	10
19th to 27th October . . .	19·5	7·6	9·2	7·6	19·5	9·2	7·6

The results reveal the same facts and the same relationships which are discussed above.

In all the above-mentioned culture experiments the strengths of the ammonium sulphate and potassium nitrate are kept constant all throughout the series. It was considered of interest to study the absorption of the $NH_4^{\cdot}$, NO_3' and SO_4'' ions from pure solutions of ammonium sulphate and potassium nitrate of different concentrations during each of the eight growth stages of the rice plant. These experiments would also determine whether the absorption of ammoniacal nitrogen decreases with age and of the nitrate nitrogen increases with age from pure solutions of ammonium sulphate and potassium nitrate as was found to be the case in the experiments described above. Concentrations of ammonium sulphate varying from $0{\cdot}005N$ to $0{\cdot}0011N$ were used. Concentrations of potassium nitrate varying from $0{\cdot}00066N$ to $0{\cdot}00143N$ were taken.

The series of experiments were so arranged that each plant remained in the solution of a single salt for a week in rotation and at the end of the week it was transferred to a normal culture solution after a day in distilled water. This was necessary as the plants could not be successfully grown in pure solution of ammonium sulphate or potassium nitrate, so they were grown in culture solution and kept in single salt solution for a week when the estimation of the $NH_4^{\cdot}$ and SO_4'' ions absorbed by the plant at a particular stage of growth had to be made. For each stage of growth more than one concentration of ammonium sulphate and potassium nitrate were used. Similarly for different concurrent stages of growth of the rice plant the same concentration of ammonium sulphate or potassium nitrate was also used.

The results of the experiments with different concentrations of ammonium sulphate at different stages of growth of the rice plants and also of rice seedlings, are given in Tables XIII and XIV. Two important conclusions can be drawn from the study of the results.

(1) The quantity of $NH_4^{\cdot}$ ions and SO_4'' ions absorbed from any one concentration of ammonium sulphate decreases as the age advances.

(2) The actual amounts of the two ions absorbed at any one stage of growth decreases as the concentration of ammonium sulphate solution is decreased but when the absorbed quantities are calculated as percentages of the initial quantities present in each solution there is an increase in the absorption of the two ions as the concentration decreases for any one stage of the plant.

In the same Tables XIII and XIV the results of the quantities of $NH_4^{\cdot}$ and SO_4'' ions absorbed are calculated as percentages of the initial quantities of the two ions present in each solution. The results show that there is an increase in the

absorption of the two ions as the concentration is decreased for any one stage of growth of the plant.

The graphs showing the percentages of absorption of NH_4' and SO_4'' ions from the three culture solutions at the successive stages of growth are shown in Figs. 1 and 2.

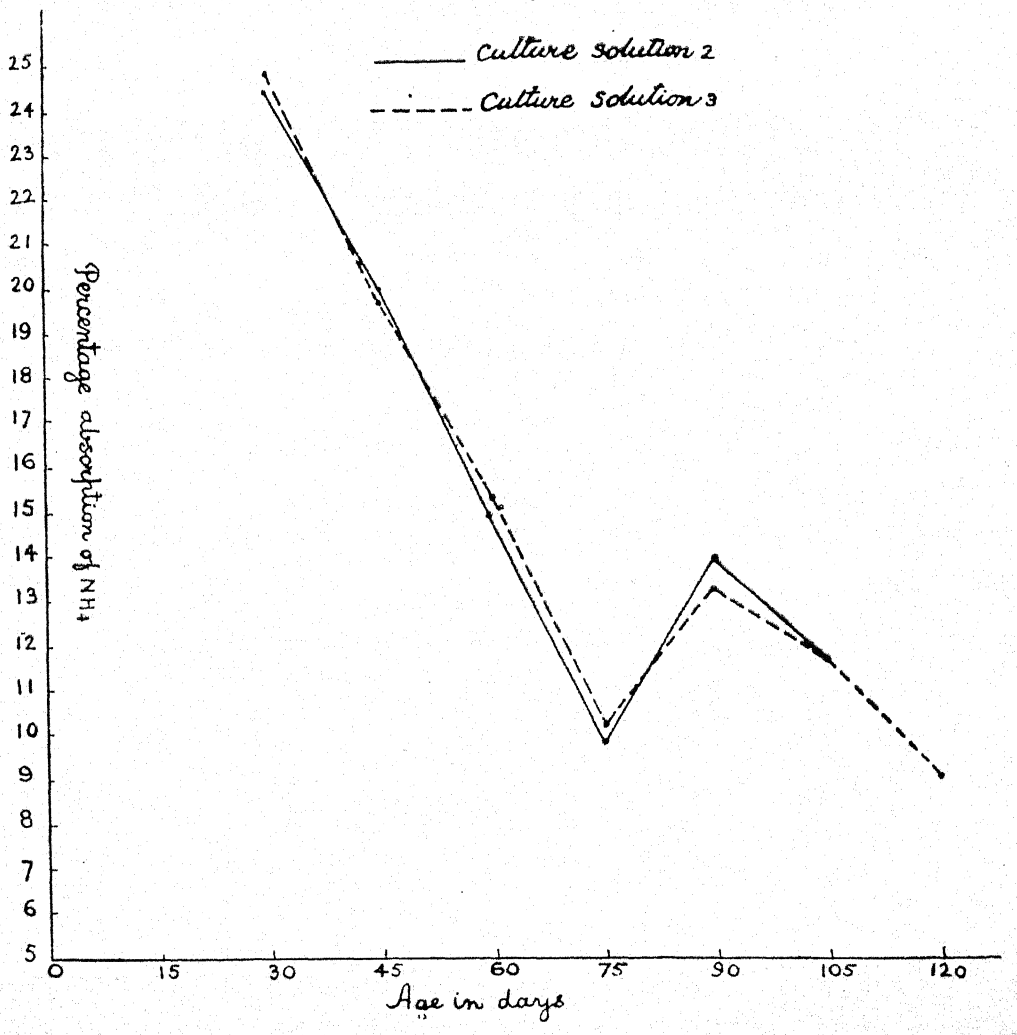

Fig. 1.

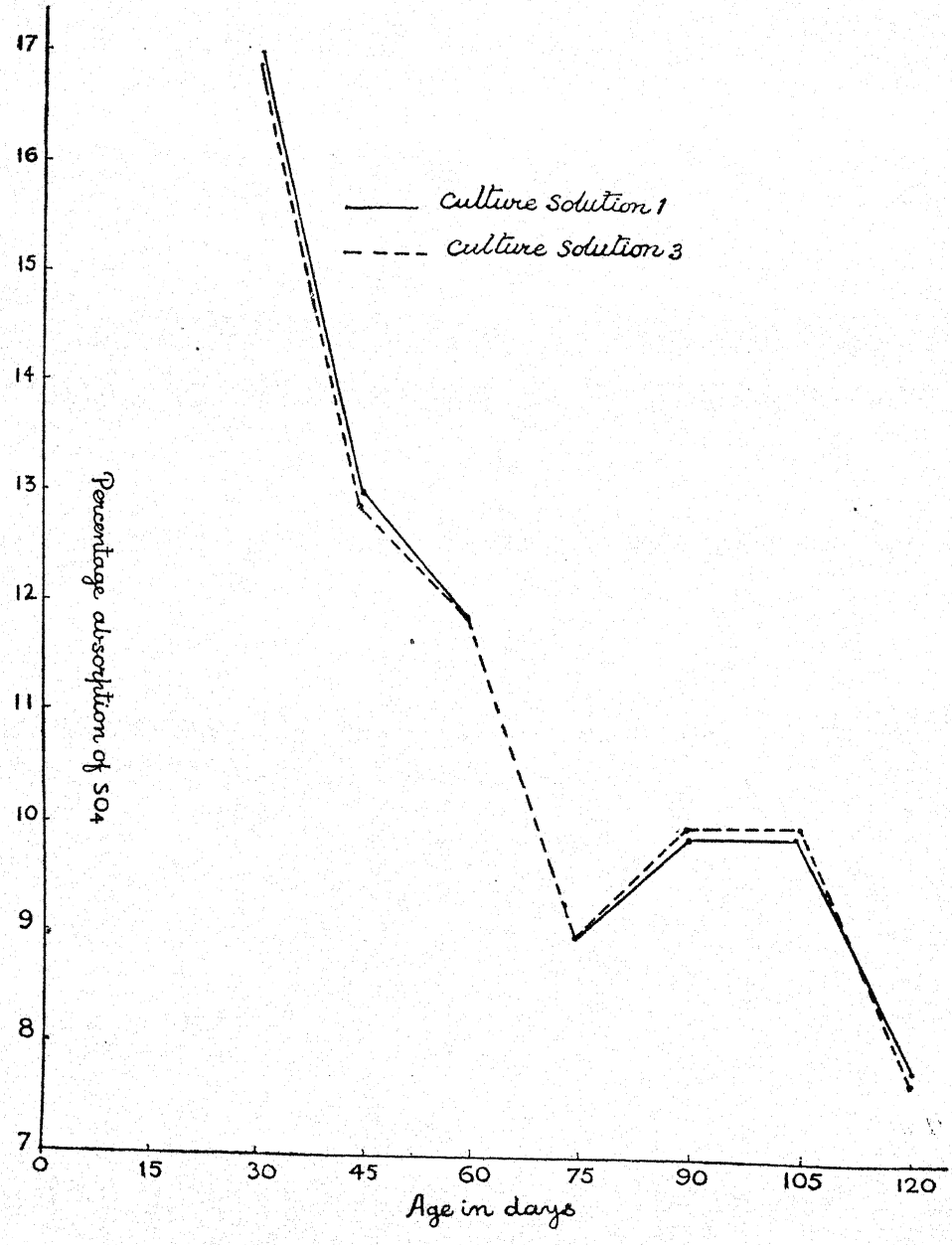
17
16
15
14
13
12
11
10
9
8
7
Culture Solution 1
Culture Solution 3
Percentage absorption of SO4
0
15
30
45
60
75
90
105
120
Age in days

Fig. 2.

TABLE XIII.

Actual and percentage absorption of ions from $(NH_4)_2 SO_4$ by rice plant.

Strength of $(NH_4)_2 SO_4$		11th July to 19th July		21st July to 29th July		10th Aug. to 18th Aug.		21st Aug. to 29th Aug.		10th Sept. to 18th Sept.		21st Sept. to 29th Sept.		11th Oct. to 19th Oct.		21st Oct. to 29th Oct.	
		NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''
$\frac{N}{200}$	Grm.	·0181	·0482	·0165	·0312	·0144	·0264	..	..	..	..	..	..		..	..	..
	Percentage	20·09	20·06	18·31	12·99	15·98	10·99	..	..	..	..	..	..	..	..	..	..
$\frac{N}{300}$	Grm.	·0169	·0448	·0144	·0272	·0120	·0208	·0084	·0128	·0060	·0096	..	..	..	..	..	..
	Percentage	28·12	27·98	23·96	16·99	19·98	12·99	13·97	7·99	9·98	5·99	..	..	..	..	..	..
$\frac{N}{400}$	Grm.	..	..	·0109	·0204	·0091	·0156	·0068	·0144	·0045	·0108	·0043	·0084	..	..	..	..
	Percentage	..	..	24·16	16·98	20·17	12·98	15·07	11·99	9·97	8·99	9·53	6·99	..	..	..	..
$\frac{N}{500}$	Grm.	..	..	·0094	·0173	·0083	·0154	·0054	·0115	·0035	·0077	·0051	·0080	·0040	·0067	..	..
	Percentage	..	..	26·11	18·07	23·05	16·02	15·00	11·96	9·72	8·01	14·16	8·32	11·11	6·97	..	..
$\frac{N}{600}$	Grm.	..	..	..	..	..	..	·0060	·0132	·0047	·0080	·0042	·0064	·0036	·0080	..	..
	Percentage	..	..	..	..	..	..	20·00	16·50	15·66	10·00	14·00	18·00	12·00	10·00	..	..
$\frac{N}{700}$	Grm.	..	..	..	..	..	..	..	..	..	..	·0041	·0069	·0020	·0069	·0023	·0052
	Percentage	..	..	..	..	..	..	..	..	..	..	15·95	10·05	11·67	10·05	8·95	7·58
$\frac{N}{800}$	Grm.	..	..	..	..	..	..	..	..	..	..	..	..	·0035	·0063	·0021	·0046
	Percentage	..	..	..	..	..	..	..	..	..	..	..	..	15·55	10·50	9·33	7·66

TABLE XIV.

Actual and percentage absorption of ions from $(NH_4)_2 SO_4$ by rice seedlings.

Strength of $(NH_4)_2 SO_4$		11th July to 19th July		21st July to 29th July		10th Aug. to 18th Aug.		21st Aug. to 29th Aug.		10th Sept. to 18th Sept.		21st Sept. to 29th Sept.		11th Oct. to 19th Oct.		21st Oct. to 29th Oct.	
		NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4'	SO_4''	NH_4	SO_4''
$\frac{N}{200}$	Grm.	·0181	·0482	·0168	·0312	·0144	·0264	..	..	..	..	..	..	..	..	..	..
	Percentage	20·08	20·06	18·64	12·99	15·98	10·99	..	..	..	..	..	..	..	..	..	..
$\frac{N}{300}$	Grm.	·0169	·0448	·0144	·0272	·0120	·0208	·0084	·0128	·0060	·0096	..	..	..	..	..	..
	Percentage	28·11	27·98	23·96	16·99	19·96	12·99	13·97	7·99	9·98	5·99	..	..	..	..	..	..
$\frac{N}{400}$	Grm.	..	..	·0109	·0204	·0091	·0156	·0068	·0144	·0045	·0108	·0052	·0072	..	..	..	..
	Percentage	..	..	24·16	16·98	20·16	12·98	15·07	11·99	9·97	8·98	11·53	5·99	..	..	..	..
$\frac{N}{500}$	Grm.	..	..	·0095	·0173	·0090	·0137	·0054	·0115	·0036	·0077	·0051	·0080	·0045	·0063	..	..
	Percentage	..	..	26·38	18·00	24·94	14·25	15·00	11·06	10·00	8·01	14·16	8·32	·1255	6·55	..	..
$\frac{N}{600}$	Grm.	..	..	..	..	..	..	·0060	·0120	·0050	·0080	·0042	·006	·0036	·0080	..	..
	Percentage	..	..	..	..	..	..	20·00	15·00	16·66	10·00	14·00	8·00	12·00	10·00	..	..
$\frac{N}{700}$	Grm.	..	..	..	..	..	..	..	..	..	..	·0041	·0062	·0033	·0069	·0023	·0052
	Percentage	..	..	..	..	..	..	..	..	..	..	15·95	9·38	12·84	10·05	8·95	7·44
$\frac{N}{800}$	Grm.	..	..	..	..	..	..	..	..	..	..	..	..	·0030	·0048	·0021	·0046
	Percentage	..	..	..	..	..	..	..	..	..	..	..	..	15·55	8·00	9·33	·66

The results of the experiments performed with different concentrations of potassium nitrate are given in Table XVI. Here the concentration of potassium nitrate solutions is increased as the plant ages as former experiments have shown that absorption of the nitrate increases with age. The results of this set of experiments with single salt solution confirm the results obtained before. The quantity of NO_3' ions absorbed from any one concentration of the potassium nitrate solution by a plant in the latter stage of growth is greater than the quantity absorbed in the earlier stages of growth. The absorption of NO_3' ions at any particular stage of growth increases as the concentration of the potassium nitrate solution is increased but after a certain concentration of potassium nitrate solution there is no further increase in the absorption of NO_3' ions.

Table XV gives the results of the experiments performed with the rice plants and Table XVI gives the results of the experiments with rice seedlings.

The same tables give the results calculated in percentages of the ions absorbed from the initial quantities present. The absorption of nitrate ion is increased from 17 per cent. to 29 per cent. from 0·0011*N* potassium nitrate solution from September to October.

The graph showing the percentage absorption of NO_3' ions from the three culture solutions at successive stages of growth is given in Fig. 3.

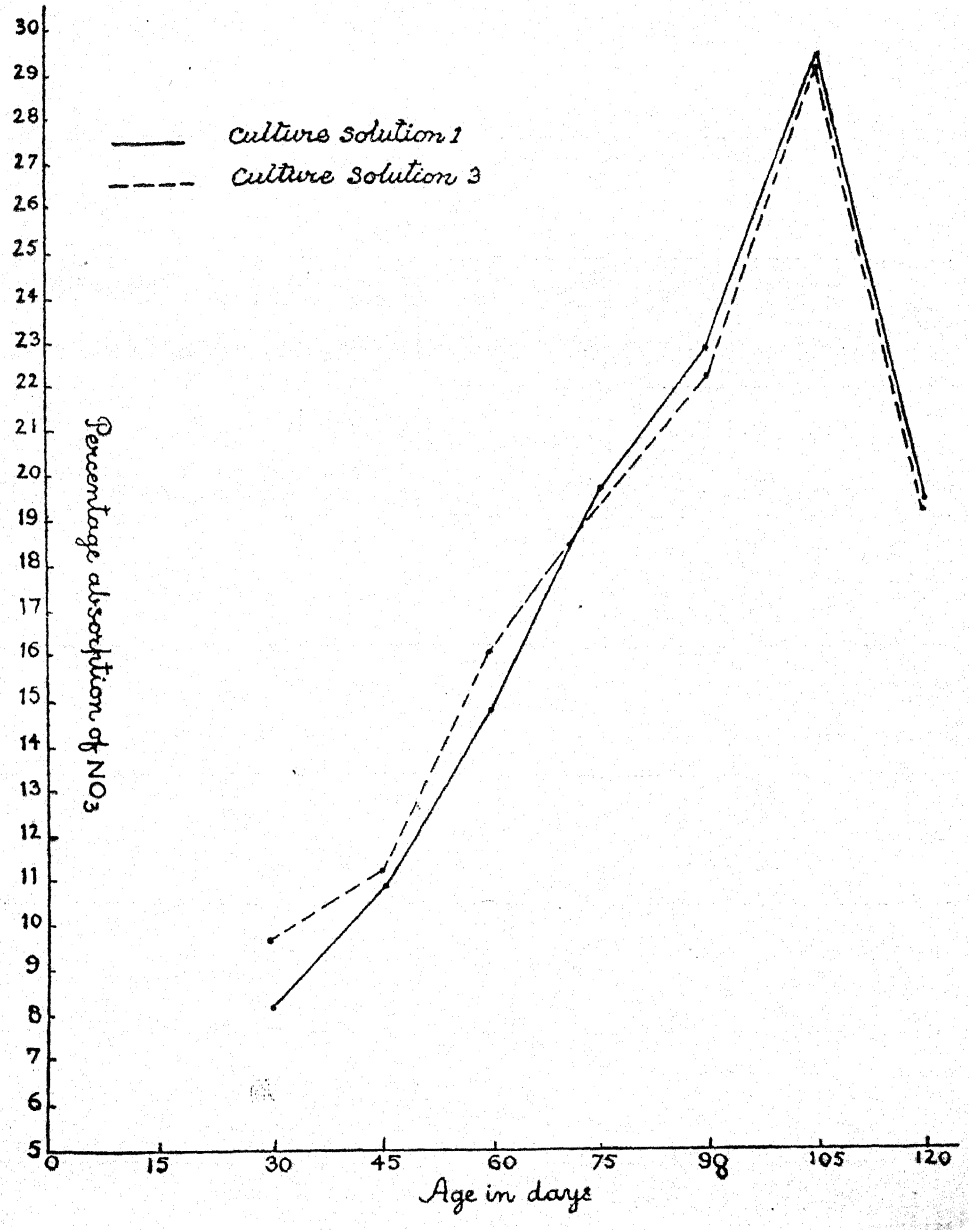

Fig. 3.

TABLE XV.

Actual and percentage absorption of NO_3' ion from KNO_3 by rice plant.

Strength of KNO_3		11th July to 19th July 15 days	21st July to 29th July 30 days	10th Aug. to 18th Aug. 45 days	21st Aug. to 29th Aug. 60 days	10th Sept. to 18th Sept. 75 days	21st Sept. to 29th Sept. 90 days	11th Oct. to 19th Oct. 105 days
$\frac{N}{700}$	Grm.	..	..	..	..	..	..	0·0222
	Percentage	..	..	..	..	..	..	25·05
$\frac{N}{800}$	Grm.	...	...	..	..	..	0·0136	0·0220
	Percentage	..	...	..	..	..	17·54	28·40
$\frac{N}{900}$	Grm.	...	..	..	..	0·0121	0·0130	0·0200
	Percentage	..	..	..	..	17·56	18·86	29·82
$\frac{N}{1000}$	Grm.	..	0·0046	0·0056	0·0093	0·0118	0·0130	0·0199
	Percentage	..	7·42	9·03	15·00	19·03	20·96	32·09
$\frac{N}{1100}$	Grm.	..	..	..	0·0090	0·0118	0·0130	..
	Percentage	..	..	..	15·98	20·96	23·06	..
$\frac{N}{1200}$	Grm.	..	..	0·0052	0·0088	0·0114	..	..
	Percentage	..	..	10·05	17·02	22·05	..	..
$\frac{N}{1300}$	Grm.	..	0·0043	0·0050	0·0081	..	..	..
	Percentage	..	9·01	10·48	16·98	..	..	..
$\frac{N}{1400}$	Grm.	..	0·0042	0·0050	..	..	..	..
	Percentage	..	9·48	11·28	..	..	..	..
$\frac{N}{1500}$	Grm.	0·0035	0·0040	..	..	..	..	..
	Percentage	8·47	9·68	..	..	..	..	..

TABLE XVI.

Actual and percentage absorption of NO_3' ions from KNO_3 by rice seedlings.

Strength of KNO_3		11th July to 19th July 15 days	21st July to 29th July 30 days	10th Aug. to 28th Aug. 45 days	21st Aug. to 19th Aug. 60 days	10th Sept. to 18th Sept. 75 days	21st Sept. to 29th Sept. 90 days	11th Oct. to 19th Oct. 105 days
$\frac{N}{700}$	Grm.	..	..	....	..	..	..	0·0222
	Percentage	..	..	..	..	..	..	25·05
$\frac{N}{800}$	Grm.	..	..	..	..	..	0·0146	0·0225
	Percentage	..	..	..	..	..	18·84	29·03
$\frac{N}{900}$	Grm.	..	..	..	..	0·0129	0·0130	0·0199
	Percentage	..	..	..	..	18·72	18·86	28·88
$\frac{N}{1000}$	Grm.	..	..	..	..	0·0118	0·0130	..
	Percentage	..	..	..	..	19·03	20·96	..
$\frac{N}{1100}$	Grm.	..	..	..	0·0090	0·0112	0·0130	..
	Percentage	..	..	..	15·98	19·89	23·09	..
$\frac{N}{1200}$	Grm.	..	..	0·0054	0·0088	0·0103	..	..
	Percentage	..	..	10·44	17·02	19·92	..	..
$\frac{N}{1300}$	Grm.	..	0·0043	0·0052	0·0080	..	..	..
	Percentage	..	9·01	10·09	16·77	..	..	..
$\frac{N}{1400}$	Grm.	..	0·0042	0·0050	..	..	..	..
	Percentage	..	9·48	11·29	..	..	..	..
$\frac{N}{1500}$	Grm.	0·0035	0·0040	..	..	..	..	..
	Percentage	8·47	9·68	..	..	..	..	..

Since nitrogen is also absorbed by the rice plant as ammoniacal nitrogen, it was also undertaken to determine the nature of ammonium salt from which the maximum quantities of ammoniacal nitrogen are absorbed. Two series of experiments like those described for the nitrate salts were arranged. The salts of ammonia used were ammonium sulphate, ammonium phosphate, ammonium chloride and ammonium nitrate. For the earlier stages of growth strong solutions of these salts were used and for later stages weaker strengths were used as it was seen that the absorption of ammonium ion decreases with the advance of age. Table XVII gives the

absorption of NH_4 ions from different salts of ammonia of the same strengths at different stages of growth.

TABLE XVII.

Actual and percentage absorption of NH_4 ion from various salts of ammonia by rice plants.

Age in days	Strength		$(NH_4)_2SO_4$	$(NH_4)_3PO_4$	NH_4NO_3	NH_4Cl
30-37	$\frac{N}{200}$	Grm.	0·0165	0·0090	0·0054	0·0052
		Percentage	18·31	9·99	5·99	5·77
45-52	$\frac{N}{200}$	Grm.	0·0144	0·0086	0·0050	0·0045
		Percentage	15·99	9·54	5·55	4·99
60-67	$\frac{N}{300}$	Grm.	0·0084	0·0048	0·0032	0·0027
		Percentage	13·97	7·99	5·32	4·49
75-82	$\frac{N}{300}$	Grm.	0·0060	0·0036	0·0025	0·0018
		Percentage	9·98	5·99	4·16	3·00
90-97	$\frac{N}{400}$	Grm.	0·0043	0·0028	0·0025	0·0018
		Percentage	9·53	6·21	6·43	4·00
105-112	$\frac{N}{500}$	Grm.	0·0040	0·0022	0·0020	0·0014
		Percentage	11·11	5·55	6·11	3·89
30-37	$\frac{N}{500}$	Grm.	0·0094	0·0054	0·0032	0·0027
		Percentage	26·11	15·00	8·89	7·50
45-52	$\frac{N}{500}$	Grm.	0·0083	0·0050	0·0030	0·0023
		Percentage	23·03	13·89	8·33	6·39
60-67	$\frac{N}{600}$	Grm.	0·0060	0·0033	0·0024	0·0021
		Percentage	20·09	4·00	8·00	7·00
75-82	$\frac{N}{600}$	Grm.	0·0047	0·0031	0·0022	0·0017
		Percentage	15·67	10·34	7·50	5·67
90-97	$\frac{N}{700}$	Grm.	0·0041	0·0023	0·0025	0·0015
		Percentage	15·95	8·95	9·73	5·84
105-112	$\frac{N}{800}$	Grm.	0·0035	0·0020	0·0018	0·0011
		Percentage	15·55	8·89	9·33	4·89

TABLE XVIII.

Actual and percentage absorption of NH_4 ion from various salts of ammonia by rice seedlings.

Age in days	Strength		$(NH_4)_2 SO_4$	$(NH_4)_3 PO_4$	NH_4NO_3	NH_4Cl
30-37	$\frac{N}{200}$	Grm.	0·0168	0·0090	0·0054	0·0052
		Percentage	18·65	9·99	5·99	5·77
45-52	$\frac{N}{200}$	Grm.	0·0144	0·0108	0·0054	0·0050
		Percentage	15·99	8·99	5·99	5·55
60-67	$\frac{N}{300}$	Grm.	0·0084	0·0048	0·0032	0·0027
		Percentage	13·97	7·99	5·32	4·49
75-82	$\frac{N}{300}$	Grm.	0·0060	0·0036	0·0025	0·0018
		Percentage	9·98	5·99	4·16	2·99
90-97	$\frac{N}{400}$	Grm.	0·0052	0·0028	0·0033	0·0018
		Percentage	11·53	6·21	7·31	3·99
105-112	$\frac{N}{500}$	Grm.	0·0045	0·0025	0·0020	0·0014
		Percentage	12·50	6·94	8·33	3·89
30-37	$\frac{N}{500}$	Grm.	0·0095	0·0055	0·0036	0·0027
		Percentage	26·38	15·28	10·00	7·50
45-52	$\frac{N}{500}$	Grm.	0·0090	0·0052	0·0029	0·0024
		Percentage	25·00	14·44	8·06	6·67
60-67	$\frac{N}{600}$	Grm.	0·0060	0·0033	0·0024	0·0021
		Percentage	20·00	11·00	8·00	7·00
75-82	$\frac{N}{600}$	Grm.	0·0047	0·0031	0·0022	0·0017
		Percentage	16·67	10·34	7·33	5·67
90-97	$\frac{N}{700}$	Grm.	0·0041	0·0023	0·0027	0·0015
		Percentage	15·95	8·95	10·51	5·84
105-112	$\frac{N}{800}$	Grm.	0·0035	0·0020	0·0016	0·0011
		Percentage	15·55	8·89	9·78	4·88

Ammonium sulphate is the best form in which ammonium ion can be supplied to the rice plant as maximum amounts of NH_4 ions are absorbed from a solution of ammonium sulphate at all stages. The different ammonium salts are in the order

sulphate, phosphate, nitrate and chloride in the early stages and as sulphate, nitrate phosphate and chloride in the later stages as far as the absorption of NH_4· is concerned. It is also evident that the absorption of NH_4· ion decreases as the plant ages, from all the ammonium salts used. It is noticed that plants kept in the solution of ammonium nitrate and ammonium chloride turned yellowish in the first two stages of growth indicating that these salts were not suitable for the healthy growth of plants in the early stages.

It was also undertaken to determine which is the best nitrate from which the maximum quantities of the NO_3' ions were absorbed and therefore the absorption of the NO_3' ions from various nitrates at different stages of growth was determined. Two series of water culture experiments were started as done before. The salts used were nitrate of potassium, sodium, calcium, magnesium and ammonium. Equimolecular solutions of all nitrates were used for each stage of growth of the rice plant. The plants were kept for eight days in each solution and then the solutions were analysed. The volume of water absorbed by the plant in a week was recorded. The amount of nitrate present in the solution, before and after the plants had been kept in the solution, was determined. The results are given in Table XIX below.

TABLE XIX.

Actual and percentage absorption of NO_3' by the rice plant from different nitrates.

Age in days	Strength of the solution		NH_4NO_3	$Mg(NO_3)_2$	KNO_3	$Ca(NO_3)_2$	$NaNO_3$
15-22	$\frac{N}{1500}$	Grm.	0·0079	0·0051	0·0035	0·0033	0·0021
		Percentage	19·13	12·35	8·47	8·01	5·08
30-37	$\frac{N}{1300}$	Grm.	0·0067	0·0048	0·0043	0·0045	0·0029
		Percentage	14·05	10·07	9·02	9·43	6·08
30-37	$\frac{N}{1000}$	Grm.	0·0074	0·0053	0·0046	0·0049	0·0031
		Percentage	12·21	8·55	7·42	7·90	5·00
45-52	$\frac{N}{1200}$	Grm.	0·0083	0·0065	0·0052	0·0058	0·0038
		Percentage	16·05	12·57	10·06	11·21	7·35
45-52	$\frac{N}{1000}$	Grm.	0·0086	0·0068	0·0056	0·0062	0·0043
		Percentage	13·37	10·96	9·03	10·00	6·94

TABLE XIX—*contd.*

Age in days	Strength of the solution		NH_4NO_3	$Mg(NO_3)_2$	KNO_3	$Ca(NO_3)_2$	$NaNO_3$
60-67	$\frac{N}{1100}$	Grm.	0·0101	0·0085	0·0090	0·0070	0·0045
		Percentage	17·94	15·13	15·99	12·46	7·99
60-67	$\frac{N}{1000}$	Grm.	0·0105	0·0039	0·0093	0·0070	0·0046
		Percentage	16·93	14·03	15·00	11·93	7·42
75-82	$\frac{N}{1200}$	Grm.	0·0152	0·0104	0·0114	0·0067	0·0044
		Percentage	29·40	20·11	22·05	12·96	8·51
75-82	$\frac{N}{1000}$	Grm.	0·0155	0·0110	0·0118	0·0081	0·0056
		Percentage	25·00	17·74	19·93	13·06	9·03
75-82	$\frac{N}{900}$	Grm.	0·0160	0·0114	0·0117	0·0090	0·0045
		Percentage	22·70	16·55	17·56	13·06	8·37
90-97	$\frac{N}{1100}$	Grm.	0·0190	0·0141	0·0130	0·0085	0·0056
		Percentage	33·75	21·31	23·09	15·01	9·95
90-97	$\frac{N}{800}$	Grm.	0·0202	0·0147	0·0136	0·0089	0·0058
		Percentage	26·07	17·03	17·55	11·48	7·48
105-112	$\frac{N}{900}$	Grm.	0·0261	0·0193	0·0199	0·0117	0·0083
		Percentage	37·37	28·02	28·89	10·98	12·05
105-112	$\frac{N}{700}$	Grm.	0·0284	0·0213	0·0202	0·0124	0·0089
		Percentage	32·05	24·04	25·06	14·00	10·05

The absorption of nitrogen from all nitrates increases as the rice plant ages. The concentration of the salt is increased as the age advances because it is found that higher concentrations at early stages injure the plants. In the later stages of growth two concentrations of each salt are used for each stage and it is seen that there is not much difference in the amounts of NO_3' absorbed from the two solutions.

The largest amount of nitrogen is absorbed from ammonium nitrate solution at all stages of growth. The different salts as regards the absorption of nitrogen stand in the order ammonium, magnesium, calcium, potassium, and sodium in the early

stages of growth and ammonium, potassium, magnesium, calcium and sodium in the later stages of growth (Fig. 4).

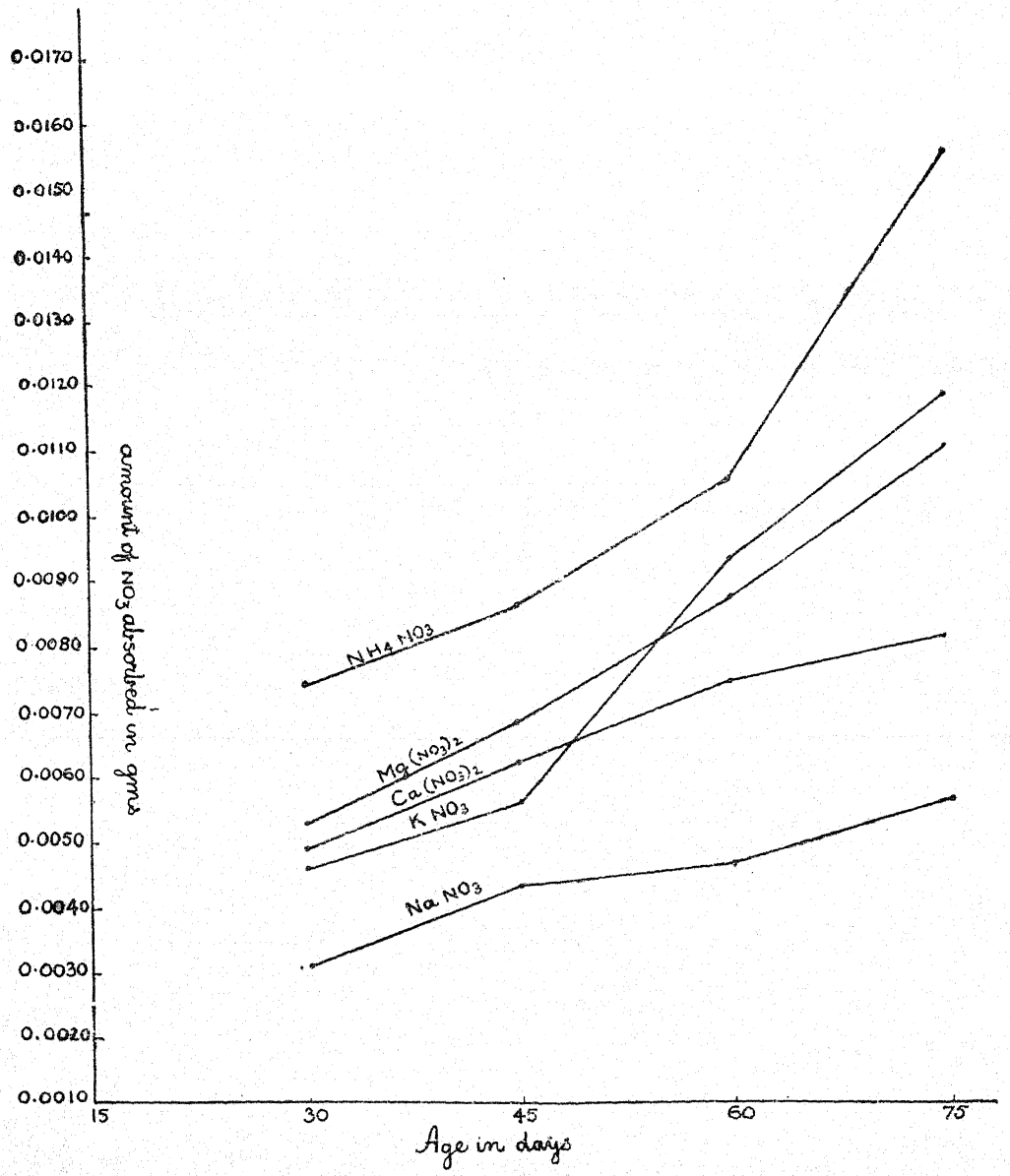

Fig. 4.

The amount of NO_3' absorbed from different nitrates is different at all stages of growth. It appears that sodium nitrate is the most unsuitable form of nitrogen to

the rice plant as very small amounts of NO_3' ions are absorbed by the rice plants at all stages of growth.

The results prove beyond doubt that nitrate ion is not taken in by the roots of the rice plants in the early stages and probably the permeability of protoplasm to the nitrate ion increases as the age advances, though there is no evidence to warrant such a conclusion. It may be argued that non-absorption of nitrate ion may be caused by the non-absorption of the positively charged ions and as the penetration of an excess of one ion cannot take place without the replacement of the excess by an equal quantity of another ion carrying the same charge, it is possible that the penetration of the nitrate ion is prevented as the positive ion of the salt is not absorbed and as there is no replacement of the NO_3' ion absorbed in excess by another ion carrying the same charge. So it was necessary to determine the absorption of both the ions from a nitrate salt by the rice plant. Potassium nitrate was selected and solutions of potassium nitrate of different concentrations varying from ·001*N* to ·00066*N* were prepared. The rice seedlings of 15 days were kept in each solution. After eight days the solutions were analysed for potassium and nitrate ions. Potassium was estimated by the gravimetric method described by Treadwell and Hall [1913] as potassium chloroplatinate and the nitrate ion as before. The results are given in Table XX.

TABLE XX.

Quantities of the potassium and nitrate ions absorbed by the rice seedlings from solutions of KNO_3.

Solution KNO_3	Initial amount in grm.		Remaining amount in grm.		Absorbed amount in grm.		Percentage of absorbed amount	
	K·	NO_3'	K·	NO_3'	K·	NO_3'	K·	NO_3'
$\frac{N}{1000}$	0·0391	0·0620	0·0316	0·0573	0·0075	0·0047	19·1	7·5
$\frac{N}{1100}$	0·0356	0·0563	0·0282	0·0518	0·0074	0·0045	20·8	8·0
$\frac{N}{1200}$	0·0326	0·0517	0·0252	0·0474	0·0074	0·0043	22·7	8·3
$\frac{N}{1300}$	0·0307	0·0477	0·0236	0·0437	0·0071	0·0040	23·1	8·4

TABLE XX—*contd.*

Solution KNO_3	Initial amount in grm.		Remaining amount in grm.		Absorbed amount in grm.		Percentage of absorbed amount	
	$K^{\cdot}$	NO_3'	$K^{\cdot}$	NO_3'	$K^{\cdot}$	NO_3'	$K^{\cdot}$	NO_3'
$\frac{N}{1400}$	0·0279	0·0443	0·0213	0·0404	0·0066	0·0039	23·6	8·8
$\frac{N}{1500}$	0·0261	0·0413	0·0196	0·0377	0·0065	0·0036	24·9	8·7
$\frac{N}{1500}$	0·0261	0·0413	0·0195	...	0·0066	...	25·0	...

The results show that the absorption of potassium ion is not at all hindered. The plant takes in potassium in greater proportion than the nitrate ion from the salt of potassium nitrate. If the results are calculated as percentages of the potassium and nitrate ions absorbed from the total quantities present in the solution it is seen that the percentage of the absorbed potassium ion increases as the concentration is decreased while the percentage of the nitrate ion absorbed remains nearly constant.

The values obtained in all the above experiments for the absorption of NO_3' ions by the rice plants at different stages of growth may have been influenced by the denitrification going on in the culture solutions. In order to make sure of this source of error in the estimations it was necessary to make tests for nitrites in the culture solutions after the plants had remained in them for eight days. The test for nitrite was done with all the culture solutions in which the rice plants of different stages of growth had been kept for a week. But in no case the nitrites were detected. The test was made according to the method of Peter Griess modified by Illosvay and Lunge [1899].

In the experiments to determine the absorption of NO_3' and $NH_4^{\cdot}$ ions from different nitrates and ammonium salts, the quantities of water absorbed by the rice plants from each solution were recorded after the plants had remained in them for eight days. On studying the amounts of water absorbed and the quantities of nitrate or ammonium ions absorbed it was found that the two quantities were correlated. It was discovered that from the solutions of nitrate salts of the same concentration the quantity of water absorbed from one nitrate solution is greater than the amount absorbed from the second nitrate salt and this greater absorption of water was also accompanied by the greater absorption of the nitrate

ion from one solution of nitrate than from the second. If more nitrate or ammonium ion passes into the plants, it was also accompanied by greater absorption of water. In order to study these relationships it is necessary that the two nitrate solutions are of the same strength and the rice plants must be in the same stage of growth in both cases. If these points are disregarded no correlationship between the amounts of water and the ions absorbed could be seen. Table XXI gives these relationships for solutions of different nitrates for the rice plants in different stages of growth.

TABLE XXI.

Relation between the amounts of water and the nitrate ion absorbed from the solutions of different nitrate salts.

Salt used	Amount of NO_3' absorbed in grm.	Percentage of the absorbed NO_3'	Quantity of water absorbed in c.c.
15 days old plants and strength of the salt solution=0·00066*N*.			
NH_4NO_3	0·0079	19·13	200
$Mg(NO_3)_2$	0·0051	12·35	170
KNO_3	0·0035	8·47	140
$Ca(NO_3)_2$	0·0033	8·01	120
$NaNO_3$	0·0021	5·08	100
30 days old rice plants, and the strength of salt solution=0·00077*N*.			
NH_4NO_3	0·0067	14·05	300
$Mg(NO_3)_2$	0·0048	10·07	200
$Ca(NO_3)_2$	0·0045	9·43	180
KNO_3	0·0043	9·02	160
$NaNO_3$	0·0029	6·08	110

TABLE XXI—*contd.*

Salt used	Amount of NO_3' absorbed in grm.	Percentage of the absorbed NO_3'	Quantity of water absorbed in c.c.
45 days old rice plants, and the strength of salt solution=0·00083*N*.			
NH_4NO_3	0·0083	16·05	350
$Mg(NO_3)_2$	0·0065	12·57	280
KNO_3	0·0052	10·06	210
$Ca(NO_3)_2$	0·0058	11·21	250
$NaNO_3$	0·0038	7·35	160
60 days old rice plants, and the strength of salt solution=0·0009*N*.			
NH_4NO_3	0·0101	17·94	370
KNO_3	0·0090	15·99	360
$Mg(NO_3)_2$	0·0085	15·13	335
$Ca(NO_3)_2$	0·0070	12·46	270
$NaNO_3$	0·0045	7·99	195
75 days old rice plants, and the strength of salt solution=0·0011*N*.			
NH_4NO_3	0·0160	23·30	490
KNO_3	0·0121	17·56	390
$Mg(NO_3)_2$	0·0114	16·55	360
$Ca(NO_3)_2$	0·0090	13·06	300
$NaNO_3$	0·0059	8·37	220

TABLE XXI—*concld.*

Salt used	Amount of NO_3' absorbed in grm.	Percentage of the absorbed NO_3'	Quantity of water absorbed in c.c.
90 days old plants, and the strength of salt solution $=0{\cdot}00125N$.			
NH_4NO_3	0·202	26·00	520
KNO_3	0·0136	17·54	400
$Mg(NO_3)_2$	0·0132	17·03	390
$Ca(NO_3)_2$	0·0089	11·48	310
$NaNO_3$	0·0058	7·48	260
105 days old plants, and the strength of salt solution $=0{\cdot}00143N$.			
NH_4NO_3	0·0284	32·05	550
KNO_3	0·0222	25·06	450
$Mg(NO_3)_2$	0·0213	24·04	420
$Ca(NO_3)_2$	0·0124	14·00	400
$NaNO_3$	0·0089	10·05	360

Similarly the greater or less absorption of NH_4· ion from the solutions of different ammonium salts is accompanied by greater or less absorption of water by the rice plants at different stages of growth as Table XXII shows.

TABLE XXII.

Relation between the amounts of water and ammonium ion absorbed from the solutions of different ammonium salts.

Salt used	Amount of NH_4· absorbed in grm.	Percentage of the absorbed NH_4·	Quantity of water absorbed in c.c.
30 days old plants, and the strength of salt solution=0·005*N*.			
$(NH_4)_2SO_4$	0·0165	18·31	360
$(NH_4)_3PO_4$	0·0090	9·99	250
NH_4NO_3	0·0054	5·99	200
NH_4Cl	0·0052	5·77	190
45 days old plants, and the strength of salt solution=0·005*N*.			
$(NH_4)_2SO_4$	0·0144	15·99	375
$(NH_4)_3PO_4$	0·0086	9·54	300
NH_4NO_3	0·0059	5·55	230
NH_4Cl	0·0045	4·99	200
60 days old plants, and the strength of salt solution=0·003*N*.			
$(NH_4)_2SO_4$	0·0084	13·97	280
$(NH_4)_3PO_4$	0·0048	7·99	230
NH_4NO_3	0·0032	5·32	200
NH_4Cl	0·0027	4·49	200

TABLE XXII—*contd.*

Salt used	Amount of NH_4 absorbed in grm.	Percentage of the absorbed NH_4	Quantity of water absorbed in c.c.
75 days old plants, and the strength of salt solution=0·0033*N*.			
$(NH_4)_2SO_4$	0·0060	9·98	300
$(NH_4)_3PO_4$	0·0036	5·99	270
NH_4NO_3	0·0025	4·16	290
NH_4Cl	0·0018	3·00	210
90 days old plants, and the strength of salt solution=0·0025*N*.			
$(NH_4)_2SO_4$	0·0043	9·53	420
$(NH_4)_3PO_4$	0·0028	6·21	350
NH_4NO_3	0·0029	6·43	380
NH_4Cl	0·0018	4·00	250
105 days old plants, and the strength of salt solution=0·002*N*.			
$(NH_4)_2SO_4$	0·0040	11·11	460
$(NH_4)_3PO_4$	0·0020	5·55	310
NH_4NO_3	0·0022	6·11	480
NH_4Cl	0·0014	3·89	280

When determining the amount of NO_3' and $NH_4^{\cdot}$ ions absorbed from the solutions of known concentration of different nitrates and ammonium salts the original and final volumes of the solutions were recorded. The volumes of the rice plants were also noted. The quantities of NO_3' and $NH_4^{\cdot}$ ions that remained unabsorbed were also determined. From these data the internal concentration and the final external concentration of each of the ion could be determined. The ratio of the two would give the absorption ratio for NO_3' or $NH_4^{\cdot}$ ion for a particular stage of growth of the rice plant for a particular concentration of any one of the salts used. The absorption ratios were then determined and it was noticed that when the same concentration of a salt is used the absorption ratio for $NH_4^{\cdot}$ ion decreases as the rice plant ages, that is, the point of equilibrium between the internal concentration and the external concentration of $NH_4^{\cdot}$ is not the same but is shifted with the increasing age of the plant. The absorption ratio $\frac{y}{c}$ for the $NH_4^{\cdot}$ ions decreases as the age of the plant advances. This is true of all ammonium salts as Table XXIII will show.

TABLE XXIII.

Absorption ratios for the $NH_4^{\cdot}$ ions.

Name of the salt	30 days old plants	45 days old plants	105 days old plants
	From 0·002 *N* solutions		
$(NH_4)_2SO_4$	8·2	3·9	1·08
$(NH_4)_3PO_4$	3·2	2·2	0·53
NH_4NO_3	1·6	1·3	0·57
NH_4Cl	1·1	0·9	0·38
	From 0·005 *N* solutions		
$(NH_4)_2SO_4$	4·77	2·96	..
$(NH_4)_3PO_4$	2·40	1·84	..
NH_4NO_3	1·50	1·05	..
NH_4Cl	1·42	1·04	..

TABLE XXIII—*contd.*

Name of the salt	60 days old plants	75 days old plants
	From 0·0033 *N* solutions	
$(NH_4)_2SO_4$	2·60	1·33
$(NH_4)_3PO_4$	1·49	0·82
NH_4NO_3	1·00	0·79
NH_4Cl	0·83	0·54
	From 0·0016 *N* solutions	
$(NH_4)_2\ SO_4$	3·30	2·04
$(NH_4)_3\ PO_4$	1·40	1·10
NH_4NO_3	1·06	0·90
NH_4Cl	0·86	0·59

Similarly the absorption ratio for NO_3' ion increases as the age of the plant advances as Table XXIV shows.

TABLE XXIV.

Absorption ratio for the nitrate ions.

Name of the salt	45 days old plants	75 days old plants
	From 0·00083 *N* solutions	
NH_4NO_3	3·1	3·3
$Mg\ (NO_3)_2$	2·5	2·9
KNO_3	2·2	2·8
$Ca(NO_3)_2$	2·2	2·5
$NaNO_3$	1·8	1·9

TABLE XXIV—*contd.*

Name of the salt	60 days old plants	90 days old plants
	From 0·0009*N* solutions	
NH_4NO_3	3·1	3·3
$Mg(NO_3)_2$	2·6	2·8
KNO_3	2·7	2·9
$Ca(NO_3)_2$	2·1	2·5
$NaNO_3$	1·6	1·8

Name of the salt	30 days old plants	45 days old plants	60 days old plants	75 days old plants
	From 0·001*N* solutions			
NH_4NO_3	3·1	3·2	3·3	3·5
$Mg(NO_3)_2$	2·2	2·5	2·6	2·8
KNO_3	2·2	2·3	2·5	2·8
$Ca(NO_3)_2$	1·8	2·1	2·2	2·4
$NaNO_3$	1·1	1·2	1·6	1·8

Name of the salt	75 days old plants	105 days old plants
	From 0·0011*N* solutions	
NH_4NO_3	3·1	3·5
$Mg(NO_3)_2$	2·3	2·7
KNO_3	2·2	2·9
$Ca(NO_3)_2$	1·8	2·7
$NaNO_3$	1·2	1·9

According to results obtained for the absorption of $NH_4^{\cdot}$ and $SO_4^{''}$ ions given in tables, it is shown that there is unequal absorption of $NH_4^{\cdot}$ and $SO_4^{''}$ ions from

solutions of ammonium sulphate, the $NH_4^{\cdot}$ ions being absorbed in larger amount than the $SO_4^{''}$ ions. The necessary consequence of the unequal absorption would be, as pointed out in the introduction, that the excess of the $NH_4^{\cdot}$ ion absorbed would be replaced either by the diffusion of positively charged ions from the roots of the rice plant or by an equivalent quantity of hydrogen ion derived from the solvent. In the latter case the excess of the absorbed $NH_4^{\cdot}$ ion would be accompanied into the roots by the hydroxyl ion and the resulting external solution would turn acidic.

It was undertaken to determine if positively charged ions diffused out from the roots to replace the excess of the ammonium ion absorbed as was found by Redfern [1922]. The tests for various positive ions like potassium, magnesium, calcium and sodium were made, but gave negative results. Thinking that the chemical tests are not sufficiently delicate to detect traces of these substances, spectroscopic analysis of the solutions used was made. Unused solutions of ammonium sulphate was examined on a spectroscope and the solution was again examined after the plants had remained in them for a week. No extra absorption lines were observed in the used solutions.

If the diffusion of positive ions from the roots did not occur the solution according to the statements made above, should turn acidic. It was therefore undertaken to determine the pH value of ammonium sulphate before and after the plants were kept in them. The determinations of pH value of the solution were made by the Indicator method and also by means of hydrogen electrode.

Determination of pH value by the Indicator method.

In the beginning the approximate pH value of a solution was determined by using universal Indicator supplied by B. D. H.

In order to obtain the correct pH value the solutions of the following indicators were prepared : thymol blue, bromo phenol blue, methyl red, bromo cresol purple, bromo thymol blue, phenol red and cresol red.

The pH value of these indicators overlap and are not in serial order so that more than one indicator could be used for determining the pH value of a solution.

Forty-eight different standard coloured solutions according to the method given by Clarke [1920] are prepared with a difference of 0·2 pH value, so that the pH value correct to 0·2 could be determined. If the matching of the colour is difficult a second indicator bromo thymol blue in this case is taken and the colour compared with the standard colour, and the pH value is again determined. The experimental error introduced in the determination of the pH value by the above method varies from $\pm$ 0·2 to $\pm$ 0·5. In some cases the magnitude of error was below $\pm$ 0·2.

All necessary precautions recommended by Clarke [1920] in making these determinations by colorimetric methods were taken. The glass vessels, and bottles used for the experiments were coated with paraffin to prevent the action of glass on solutions. It was noticed that the pH value of solution changes on being allowed to stand in glass jars.

The pH values of four different strengths of each salt in solution were first determined.

Rice plants of different stages of growth were then kept in some of these solutions and the pH values of the solution after eight days were again determined. In the earlier stages of growth $NH_4^{\cdot}$ ions absorbed are more than the corresponding negative ions from the different ammonium salts so the solutions must turn more acidic after the plants had remained in them than the unused solutions. Secondly as the absorption of $NH_4^{\cdot}$ ion decreases as the plant ages, the change in the pH value of the solution should be less when older plants are kept than when the plants in earlier stages of growth are used.

The results obtained agree with the theoretical expectations.

TABLE XXV.

Showing pH values of the solutions used.

Solutions	pH value of the original solution	pH value of the solution after keeping plants 30 days old	pH value of the solution after keeping plants 45 days old	pH value of the solution after keeping plants 75 days old	pH value of the solution after keeping plants 90 days old
$\frac{N}{200}$ $(NH_4)_2SO_4$ . .	5·0	3·0	3·2	4·0	4·0
$\frac{N}{500}$ $(NH_4)_2SO_4$ . .	5·2	2·8	3·0	4·0	4·0
$\frac{N}{200}$ $(NH_4)_3PO_4$ . .	4·2	3·4	3·4	4·0	4·0
$\frac{N}{500}$ $(NH_4)_3PO_4$ . .	4·2	3·2	2·2	4·0	4·0
$\frac{N}{200}$ NH_4Cl . . .	5·0	3·6	3·6	4·0	4·0
$\frac{N}{500}$ NH_4Cl . . .	5·2	3·4	3·6	4·0	4·0
$\frac{N}{200}$ NH_4NO_3 . . .	5·0	4·0	3·8	6·0	6·0
$\frac{N}{500}$ NH_4NO_3 . . .	5·2	3·8	3·6	6·0	6·0

The results show that the solutions of all the ammonium salts turn acidic after the rice plants had remained in them for eight days.

The change in the pH value of the solutions after the plants had remained in them is not due to the secretion of acidic substances or evolution of carbon dioxide. This is tested by experiment. The fall in the pH value is greater when the rice plants 30 days and 45 days old are used than when the older plants of 75 days and 90 days are used. In the ammonium nitrate solution the pH value rises when the plants of 75 and 90 days are kept in the solutions. This is so because the NO_3' ion absorbed increases as the plant ages.

The changes in the pH values of the solutions of different nitrates were also noted for the corresponding growth stages of the rice plants. The results are given in Table XXVI.

TABLE XXVI.

Showing pH values of the solutions of nitrate salts used.

Solution	pH value of the original solution	pH value after keeping plant 30 days old	pH value after keeping plant 45 days old	pH value after keeping plant 75 days old	pH value after keeping plant 90 days old
$\frac{N}{1300}$ KNO_3 . . .	5·8	4·4	4·4	6·0	6·0
$\frac{N}{1300}$ $Mg(NO_3)_2$ . .	5·8	4·2	4·2	6·2	6·0
$\frac{N}{1300}$ $Ca(NO_3)_2$ . .	5·8	4·2	4·2	6·2	6·0
$\frac{N}{1300}$ $NaNO_3$ . . .	5·8	4·6	4·4	6·2	6·2
$\frac{N}{1300}$ NH_4NO_3 . . .	5·3	4·0	4·0	6·0	6·0

In the early stages of growth the positive radical is more absorbed than the nitrate ion. This is shown to be the case by the analysis of the $K^{\cdot}$ and NO_3' ions of different concentrations of potassium nitrate solution in Table XX, and the solutions turn acidic. In the later stages of growth the nitrate ion is absorbed in greater proportion than the positive radical and so the rise in the pH value of the solution occurs.

CONCLUSIONS.

Some facts about the intake of nitrogen not known before, have been brought to light by the investigations described above. There is an equal absorption of

the anion and kation from ammonium sulphate, the $NH_4^{\cdot}$ ions being absorbed in greater amounts than the sulphate ions. As there is no exosmosis from the roots of positively charged ions to replace the excess of $NH_4^{\cdot}$ ions absorbed, the hydrogen ions of the solvent replace the excess of $NH_4^{\cdot}$ ions absorbed and this is confirmed by the determination of the pH values of ammonium sulphate solution before and after use.

The absorption of $NH_4^{\cdot}$ ion decreases as the rice plant grows and the quantity of $NH_4^{\cdot}$ ion absorbed in the later stages is comparatively very little. The fact explains the conclusions of Kellner [1884] and others that ammonium sulphate has a low value as a fertilizer during the later stages of growth of the rice plant.

The intake of nitrate nitrogen by the rice plant is in the reverse order, as nitrates are taken in small quantities in the earlier stages and their absorption increases as the rice plant ages. On account of the selective absorption of nitrate nitrogen Kellner [1884] and others found that rice plants showed better growth when supplied with nitrate in the later stages of growth.

The absorption of ammoniacal nitrogen and the non-absorption of nitrate nitrogen in the early stages and the reversed state of things during the later stages are points of great importance.

It is not improbable that the permeability of protoplasm may be responsible for the absorption of one ion at one stage and of the other at the other stage of growth. Though there is no direct evidence to support such a conclusion the selective absorption of ammoniacal nitrogen at one stage and of nitrate nitrogen at another stage points to such a possibility. The $NH_4^{\cdot}$ ions carry a positive charge and NO_3' ions carry a negative charge and the difference in the electric charges carried by these ions may make one ion more permeable to protoplasm than the other at a particular stage of growth. Further investigation is necessary to prove the above conclusion.

Another important fact is brought to light by the determination of the absorption ratios of the $NH_4^{\cdot}$ and NO_3' ions. The quantity of $NH_4^{\cdot}$ or NO_3' ions taken in by a plant depends upon the point of equilibrium reached between the external and internal concentrations of an ion. The point of equilibrium is not necessarily reached when the ionic concentrations outside and inside are equal. In the case of the rice plant the point of equilibrium is reached after 8 days and the internal concentration of $NH_4^{\cdot}$ ion is higher than the concentration of $NH_4^{\cdot}$ in the external solution. (This does not suggest that the $NH_4^{\cdot}$ ions remain as $NH_4^{\cdot}$ ions within the plant after being absorbed but since further absorption of $NH_4^{\cdot}$ ion ceases after eight days, it is taken that the point of equilibrium is reached. Since there is no means of finding out the amount of $NH_4^{\cdot}$ ions converted to something else from the quantity of $NH_4^{\cdot}$ ion absorbed, the total quantity absorbed

is taken as the $NH_4^{\cdot}$ ion concentration of the rice plant. Stiles and Kidd [1919] also have followed the same rule).

As the absorption ratio for the $NH_4^{\cdot}$ ions falls as the plant ages the rice plant will absorb much less $NH_4^{\cdot}$ ions at a later stage than it can absorb at an earlier stage from the same concentration of ammonium sulphate solution. This fact is important as a greater proportion of ammonium sulphate, if supplied in large dozes at later stages of growth, will remain unutilized by the rice plant as much of it will remain unabsorbed on account of the low absorption ratio. Similarly a large proportion of nitrate will remain unabsorbed, if supplied at early stages of growth, as the ratio of the nitrate absorbed to the nitrate remaining unabsorbed from more concentrated solution will be smaller than the corresponding ratio of the two quantities from less concentrated solutions. In other words percentage absorption of the $NH_4^{\cdot}$ ion at later stages is greater from the less concentrated solution than from the more concentrated solution. It is possible that the low value of ammonium sulphate as a fertilizer in the later stages of growth is not due to the lack of assimilation of ammoniacal nitrogen but to its non-absorption.

It is known that the quantity of nitrogen taken up by the rice plant is very little as compared to the quantity supplied to the soil in the form of fertilizers and the results discussed above give a reasonable explanation of the fact. The quantity of nitrogeon absorbed by the rice plant, if other changes in the soil are not considered, will vary according to the form of nitrogen supplied and of the stages of growth at which it is supplied. It is also seen that the absorption of nitrogen in any of the forms is not hindered by the presence of any other ions present. The results can have a practical application. As the absorption of $NH_4^{\cdot}$ ion decreases and the absorption of NO_3' ion increases as the plant ages, greater absorption of nitrogen will occur at any stage of growth when a mixture of the ammonium sulphate and a nitrate is supplied than when any one of them is supplied singly with the same quantity of total nitrogen as supplied in the mixture.

As nitrogen is an important element for the plant growth, it is expected the plant will show better growth when treated with mixture than when treated with ammonium sulphate or a nitrate. The greatest differences in growth will also be obtained if the mixture is supplied at a time when the plant is passing through the period of maximum growth rate.

These conclusions are being put to a practical test at present.

Summary.

It is undertaken to study the intake of nitrogen in the form of ammoniacal nitrogen and nitrate nitrogen by the rice plant at different stages of growth. The Columba variety No. 42 of the rice plant is used in this investigation. The rice

seedlings are grown in water culture solutions from the beginning of the germination of the seeds, and rice plants at the transplantation stage and from the soil are also kept in culture solutions and are used as a duplicate series. The quantity of an ion absorbed by the plant is determined by chemical analysis of the solution for that ion before and after use.

(2) From a solution of ammonium sulphate the rice plant absorbs greater quantity of $NH_4^{\cdot}$ ions than the sulphate ions. This is found to be true at all stages of growth. The absorption of $NH_4^{\cdot}$ ion decreases as the plant ages.

The absorption of $NH_4^{\cdot}$ ion is independent of the presence and absence of nitrate ion or any other ion as the same quantity of $NH_4^{\cdot}$ ions is absorbed from all solutions used at a particular stage.

As a result of unequal absorption of the $NH_4^{\cdot}$ and $SO_4^{''}$ ions from ammonium sulphate solutions the latter should turn more acidic. This has been found to be the case by determining the pH value of the ammonium sulphate solution before and after use. Determinations of pH values are made by the Indicator method given by Clarke [1920].

The absorption ratios of $NH_4^{\cdot}$ ions at different stages of growth are determined. The absorption ratio of $NH_4^{\cdot}$ decreases as the plant ages.

It was also undertaken to determine a salt of ammonia from which the maximum quantity of ammonium ion is taken. Ammonium sulphate, nitrate, chloride and phosphate were tried and the absorption of $NH_4^{\cdot}$ ion was determined from the solution of different concentrations of these salts at different stages of plant growth and it was found that the salts stood in the order, sulphate, phosphate, nitrate, and chloride in the earlier stages of growth, and sulphate, nitrate, phosphate and chloride in the later stages of growth as far as the absorption of ammonium ion was concerned.

The absorption of nitrate ion increases as the plant ages. The non-absorption of NO_3' ion from a solution of nitrate is not caused by the non-absorption of the positive ion as the results with potassium nitrates show. The absorption of NO_3' ion is independent of the presence or absence of other foreign ions.

The absorption ratios of the NO_3' ions at different stages of growth are determined, and it is found that the absorption ratios increase as the plant ages. It was also undertaken to determine the nitrate from which the maximum quantity of NO_3' ion is absorbed; nitrates of potassium, sodium, magnesium, calcium and ammonia were tried. The absorptions of NO_3' ions from the solutions of different concentrations of the salts were determined at different stages of growth and it was found that the salts stood in the order of ammonium, magnesium, calcium, potassium, and sodium in earlier stages of growth, and ammonium, potassium, magnesium, calcium and sodium in later stages of growth, when the percentage absorption of the NO_3' ion was considered.

The reason of greater absorption of NH_4' ion at early stages and of NO_3' ion at later stages may be assigned to the differences in the permeability of protoplasm at different stages of growth.

The low value of ammonium sulphate as a fertilizer and the high value of nitrate at later stages of growth found by Kellner [1884] and others can be explained by the results obtained above.

The absorption of nitrogen by the rice plant according to the results obtained, depends upon the form in which nitrogen is supplied and upon the stage of growth at which it is given.

It can also be concluded that a mixture of ammonium sulphate and potassium nitrate will form a better source of nitrogen to the rice plant than any one of them used singly with the same total nitrogen that is present in the mixture. As nitrogen is an important element, it is suggested that the plants supplied with a mixture of two forms of nitrogen will make better growth than the plants treated with any one of them.

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THE INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.)—THE ITALIAN MILLET.

PART III. BRISTLES.*

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(With Plate XV)

Setaria italica, the Italian millet, is popularly called in Western countries the fox-tail millet. This name is in consequence of the common varieties of this millet having the general appearance of a fox's tail. This fox-tail look to this millet arises through the existence of floral structures called bristles constantly present in the *Setarias*. Bristles when long enough give the fox-tail look. The shorter ones look otherwise. These bristles and the differences among them afford considerable aid in the classification and recognition of cultivated varieties. With the help of this easily-visible character, the purity of strains can be maintained and natural crosses promptly rogued out. Proper parents may be chosen for hybridization work with the certainty of detecting the success of crosses. The inheritance of this bristle character is presented in this article.

* Part I (Grain Colours) and Part II (Anther Colours) have appeared in this Journel, Vol. I, Part V and Vol. II, Part I, respectively.

Much speculation has centered round the origin and significance of these bristles. It is needless here to enter into these. Suffice it to say that these bristles exist in every head of the Italian millet subtending the grain (spikelet). Comparative studies in the floral structure of sister *Setarias* and the fact that some of these bristles have occasionally a spikelet perched at their tip lends evidence to their being aborted parts of a fascicle.

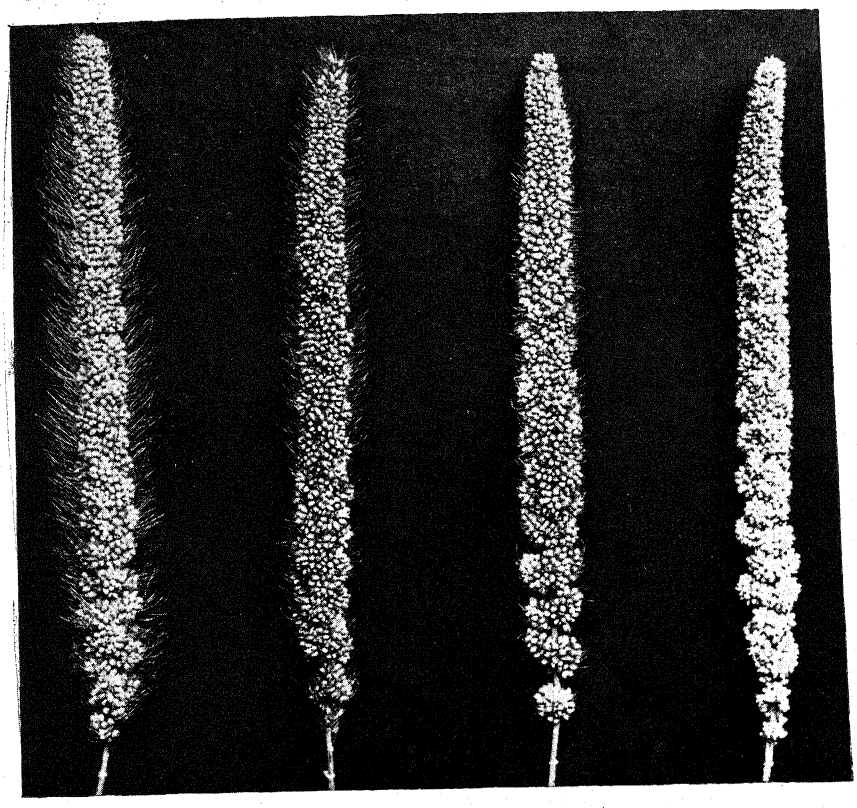
These bristles are usually one in number and often two. Three and four are rarely met with. In distribution the largest are the ones. Numbers up to nine referred to by Gammie [1911] were not met with in the varieties studied.

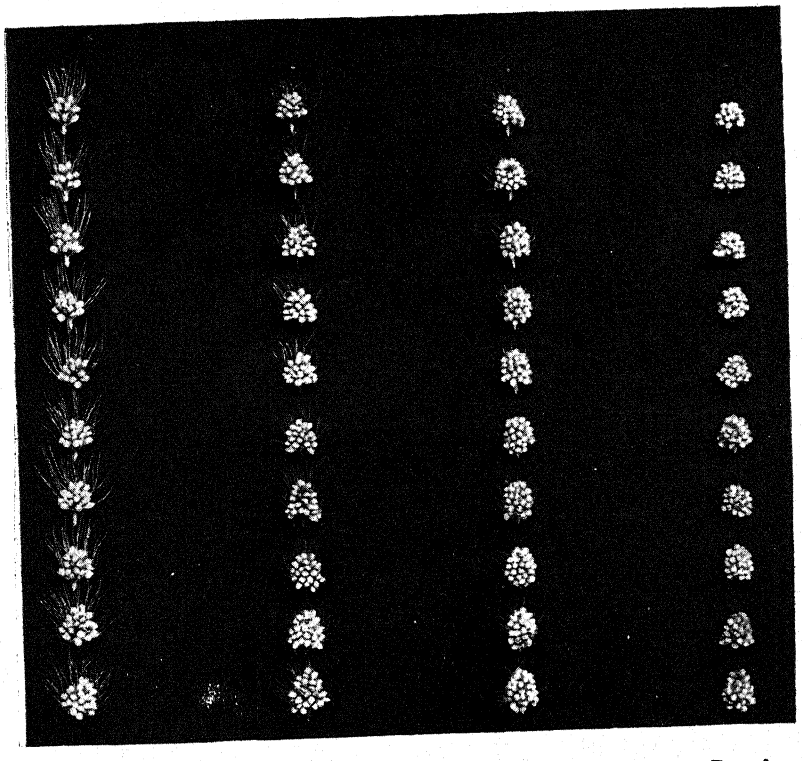
These bristles vary in their length. The easiest separation is into long and short, and this has been recognised and pursued in inheritance by earlier workers, 'Long' being found to be dominant to 'short' [Saito. 1923]. Deeper work, larger experience and the marshalling of more extensive data leave a doubt as to the finality of such a simple disposal as the above. It has been found that the delimitation of groups in pure lines was no simple matter, but implemented with the study of segregations met with, the separation of bristles into four groups was attempted with success.

The first group is the 'long'. This varies from 7 to 14 mm. in length. The average length of the group is 10·5 mm. The second group is the 'medium'. The range of this is from 4·5 to 9 mm. with an average of 8 mm. The third is 'short', ranging from 3 to 7 mm., with an average of 5 mm. The last and basic group is the 'dwarf', with a range of 2·2 to 4·5 mm. and an average of 3·2 mm. These are figured as they occur in the earhead and in individual spikes (Plate XV).

The method pursued in the grouping of these bristles is to take off six spikes from the earhead at equal distances from top to bottom, tease these spikes, note down their composition and measure off the length of the bristles in them. Individual bristle lengths, spike-average and head-averages are thus obtained. The sum total of this experience gives the average net energy in the particular variety for the expression of bristles. Two-hundred-and-fifty heads were thus examined and over 30,000 readings taken.

Bristles being protuberances beyond the grain line in an earhead, the background for the reading of these bristles is of great practical importance. It is a matter of common knowledge that earheads vary in density and though this factor adds to the difficulties in bristle groupings, yet with a little experience and study *en masse* the genetic individuality and consequent separation of the groups gets to be a fairly easy matter. In the earlier years of the work, without the support of such experience a finer separation was not possible, but later selections and restricted segregations afforded workable limits and group standards, with the





Long. Medium. Short. Dwarf.

BRISTLES.

help of which the pursuit of the major recognisable genetic groups was made possible.

All *Setarias* have bristles. The lowest expression of the bristle is 'dwarf', in which the tips of a few bristles just peep out of the undulating grain surface of the earhead. This group is best detected by holding the earhead against light and letting the bristle tips stand out of the head contour. This basic presence of the bristle is set down to the existence of a factor X.

This factor may exist by itself or in association with factor E whose existence determines the expression, both potential and actual, of the other factors contributing to length. The phenotypic expression of the 'dwarf' bristle being by itself minute, differences in expression between Xe and XE are impractical of pursuit. In the few examined, no significant differences were noticeable. That the E factor exists is only demonstrable through genetic experience.

Two factors designated L_1 and L_2 are present and act upon the basic factors Xe and XE. L_1 acting on Xe gives a 'short' bristle. L_2 acting on Xe gives a 'short' bristle also, both the 'shorts' being practically inseparable. When both L_1 and L_2 act on Xe the bristle is 'medium' in length. L_1 acting on XE produces only a 'short' bristle. L_2 acting on XE produces a 'medium' bristle. L_1 and L_2 acting conjointly on XE produce a 'long' bristle. Whereas factors L_1 and L_2 represent two doses of factors for length, their effect varies according as they act with or without the aid of the factor E which determines their full expression. Without E they produce 'short' and 'medium'. With E they result in 'short', 'medium' and 'long'. There are thus four phenotypic expressions of eight genotypes.

The experiences from over 180 S. I. (*Setaria italica*) families are presented below in support of the above hypothesis.

S. I. 296 clan is the premier clan in this experience and its history has been detailed in Table I. A natural cross in 1926, it has been continued up to the sixth generation and it provides a fairly complete and conclusive testimony for the explanation advanced above. Being one of the earlier clans, lack of experience and clear group standards inevitably led, in the first years, to a grouping together of separable bristle classes. Wherever it was necessary to present such a combination, the figures are entered midway between the columns for the respective bristle groups.

Unelaborated as it is, the F_2 figures are a good approximation to the theoretical expectation on a three-factor hypothesis; as also the F_3 figures. The further generations give a simplified ramification of the 9 : 3 : 3 : 1 experience. Unfortunately the F_5 of this clan suffered badly on account of heavy rains and beyond noting the segregations for bristle groups it was thought unsafe to pursue them in numbers, running the risk of damaged bristles leading to dubious groups. How-

ever, further careful selections of undoubted types were carried forward to F_6 which has vindicated the expectations based on the earlier experience of the clan.

TABLE I.

Clan S. I. 296. (Natural Cross spotted 1926). Segregating for E, L_1 and L_2.

Generation and family		Character of selection, constitution, and expected segregation	Bristle groups			
			Long	Medium	Short	Dwarf
F_2	S. I. 296	Long . . $EeL_1l_1L_2l_2$ (27 : 18 : 15 : 4)	64	37		39
F_3	S. I. 911, 912	Long . . $EeL_1l_1L_2l_2$ (27 : 18 : 15 : 4)	231	159	127	25
	S. I. 910, 922	Long . . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	302	227		23
	S. I. 919	Long . . $EeL_1L_1L_2l_2$ (9 : 3 : 4) or $EeL_1l_1L_2L_2$ (9 : 6 : 1)	135	110		..
	S. I. 913, 914, 923	Medium . $Eel_1l_1L_2l_2$ (9 : 3 : 4) or $eeL_1l_1L_2l_2$ (9 : 6 : 1)	..	478	329	
	S. I. 925, 926	Medium . $Eel_1l_1L_2L_2$ $eeL_1l_1L_2L_2$ or $eeL_1L_1L_2l_2$ (3 : 1)	..	355	114	..
	S. I. 915, 918, 920, 921	Short . . $EeL_1l_1l_2l_2$ $EEL_1l_1l_2l_2$ $eeL_1l_1l_2l_2$ or $eel_1l_1L_2l_2$ (3 : 1)	..	..	800	308
	S. I. 927	Short . . $EEL_1L_1l_1l_2$ $eeL_1L_1l_2l_2$ or $eel_1l_1L_2L_2$	..	..	Pure	..
	S. I. 916, 917, 924	Dwarf . $EEl_1l_1l_2l_2$ or $eel_1l_1l_2l_2$	..	..	..	Pure
F_4	*From S. I. 910.* S. I. 1215, 1218, 1219, 1222	Long . . $EEL_1L_1L_2L_2$	Pure	..	..	..

TABLE I—*contd.*

Generation and family	Character of selection, constitution, and expected segregation	Bristle groups			
		Long	Medium	Short	Dwarf
S. I. 1212 1213 1214 1216 1217 1220	Long . . $EEL_1l_1L_2L_2$ (3 : 1)	1457	535	..	..
S. I. 1211 1221	Long . . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	390	135	127	44
F_5 *From S. I. 1211.*					
S. I. 1786 1789 1801	Long . . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	x	x	x	x
S. I. 1794	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	x	..	x	..
S. I. 1810	Short . . $EEL_1l_1l_2l_2$ (3 : 1)	..	..	x	x
F_6 *From S. I. 1786, 1789 and 1801.*					
S. I. 2078 2088	Long . . $EEL_1L_1L_2L_2$	Pure	..	..	..
S. I. 2066 2076 2089 2093 2094	Long . . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	340	109	132	42
S. I. 2069 2077 2091 2092	Long . . $EEL_1l_1L_2L_2$ (3 : 1)	269	92	..	..
S. I. 2064 2065 2067 2068 2079 2095 2096	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	418	..	134	..
S. I. 2097	Medium . $EEl_1l_1L_2l_2$ (3 : 1)	..	89	..	32
S. I. 2070 2071 2072 2074 2080 2098 2099 2100	Short . . $EEL_1l_1l_2l_2$ (3 : 1)	..	..	520	229

TABLE I—*concld.*

Generation and family	Character of selection, constitution, and expected segregation	Bristle groups			
		Long	Medium	Short	Dwarf
S. I. 2081	Short . . $EEL_1L_1l_2l_2$	..	..	Pure	..
S. I. 2073	Dwarf . $EEl_1l_1l_2l_2$	..	..	..	Pure
2075					
F_6 *From S. I. 1794.*					
S. I. 2083	Long . . $EEL_1L_1L_2L_2$	Pure	..	..	..
2084					
2085					
S. I. 2082	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	50	..	14	..
S. I. 2086	Short . . $EEL_1L_1l_2l_2$	..	..	Pure	..
2087					
F_6 *From S. I. 1810.*					
S. I. 2107	Short . . $EEL_1L_1l_2l_2$	..	..	Pure	..
2112					
2115					
S. I. 2108	Short . . $EEL_1l_1l_2l_2$				
2109	(3 : 1)	..	..	300	115
2110					
2111					
2113					
2114					
S. I. 2116	Dwarf . $EEl_1l_1l_2l_2$	..	..	..	Pure
2117					
2118					
2119					
2120					

In Table II is presented the behaviour of clan S. I. 755, which seconds a restricted phase of the S. I. 296 experience given above.

TABLE II.

Clan S. I. 755. (*Natural Cross spotted in 1927*). *Segregating for E, L_1 and L_2.*

Generation and family	Character of selection, constitution, and expected segregation	Bristle groups			
		Long	Medium	Short	Dwarf
F_2 S. I. 755	Long . $EeL_1l_1L_2l_2$ (27 : 37)	58		67	
F_3 S. I. 1088	Long . $EeL_1l_1L_2l_2$ (27 : 18 : 15 : 4)	107	76	50	3
S. I. 1089 1090	Long . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	270	88	93	32
F_4 From S. I. 1088					
S. I. 1775	Long . $EeL_1l_1L_2l_2$ (27 : 18 : 15 : 4)	81	73	51	16
S. I. 1754	Medium . $EeL_1l_1L_2L_2$ $eeL_1l_1L_2L_2$ or $eeL_1L_1L_2l_2$ (3 : 1)	...	228	81	...
S. I. 1751	Medium . $EeL_1l_1L_2l_2$ (9 : 3 : 4) or $eeL_1l_1L_2l_2$ (9 : 6 : 1)	...	119	102	
S. I. 1753 1756	Short . $EEL_1l_1l_2l_2$ $eeL_1l_1l_2l_2$ or $eel_1l_1L_2l_2$ (3 : 1)	...	...	295	135

A striking feature of the bristle segregation in *Setaria* is the occurrence of the four bristle groups in the two different proportions, 27 : 18 : 15 : 4 and 9 : 3 : 3 : 1. This has been experienced above. Another important feature is the occurrence of 'long', 'medium' 'short' and 'dwarf' according to the 9 : 3 : 3 : 1 ratio as also a 9 : 3 : 4 of 'long', 'medium' and 'short'. This was the problem as well as a solution for the bristle situation in *Setaria*. But the *modus operandi* of the bristle genes being what it is, these experiences are not only possible but inevitable. If the parent is homozygous for the E factor but heterozygous for the other two length

factors, the segregation will be of a dihybrid type, and the final term of the ratio containing E will produce a 'dwarf.' Such an experience from two natural crosses is detailed in Table III. This is also supported by artificial crosses.

TABLE III.

Pure for E and segregating for L_1 and L_2.

Generation and family	Character of selection, constitution, and expected segregation	Bristle groups			
		Long	Medium	Short	Dwarf
F_2 S. I. 1940 1941 *Artificial cross LXIV and LXV*	Long . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	47	17	13	6
S. I. 1263	... $EEl_1l_1L_2L_2$	...	♀	...	...
S. I. 1264	... $EEL_1L_1l_2l_2$	...	...	♂	...
F_1 S. I. Cross LXIV LXV	Long . . $EEL_1l_1l_2l_2$	F_1	...	...	...
F_2 I. 1935 1936 1937 1938 1939	Long . $EEL_1l_1L_2l_2$ (9 : 3 : 3 : 1)	265	86	90	27

If, on the other hand, the L_1 factor be held homozygous, the final term of the ratio contains L_1 and, *ex hypothesi*, it is a 'short' producer. Hence the absence of 'dwarf' and the resultant 9 : 3 : 4 ratio (Table IV). In fact the reconciliation of these two phenomena is the central problem of bristle inheritance in this millet. The above hypothesis brings together the phenomena and in both the cases artificial crosses confirm it.

TABLE IV.

S. I. Cross XLIX, L, and LII. Pure for L_1 and segregating for E and L_2. (1930)

Generation and family	Character of selection, constitution, and expected segregation	Bristle groups		
		Long	Medium	Short
S. I. 316	.. $eeL_1L_1l_2l_2$	..	..	♂
S. I. 301	.. $EEL_1L_1L_2L_2$	♂	...	...
F_1 S. I. Cross XLIX L LII	.. $EeL_1L_1L_2l_2$	F_1	...	..
F_2 S. I. 1925 1926 1927 1928 1929	Long . . $EeL_1L_1L_2l_2$ (9 : 3 : 4)	149	46	59

The tables that follow give the segregations which are the natural corollaries of the scheme of inheritance detailed above. The 'shorts' though phenotypically inseparable are nevertheless of two genotypes. The segregation of a type like $eeL_1l_1L_2l_2$ gives a 9 : 6 : 1, a possible though not a very common Mendelian ratio. In Table V such an experience is recorded.

TABLE V.

(*Natural Crosses spotted 1926*). *Pure for e and segregating for* L_1 *and* L_2.

Generation and family	Character of selection, constitution and expected segregation	Bristle groups		
		Medium	Short	Dwarf
F_2 S. I. 750, 756, 977	Medium . . $eeL_1l_1L_2l_2$ (9 : 6 : 1)	287	204	30

Tables VI to IX show the simpler aspects of the bigger segregation chronicled above.

TABLE VI.

Families S. I. 164, 157, and 196. Segregating for 'long' and 'short'. Pure for E, L_1 *and segregating for* L_2.

Season	Generation and family	Character of selection, constitution, and expected segregation	Bristle groups	
			Long	Short
1925	F_2 S. I. 164	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	53	19
1926	F_3 S. I. 263, 266	Long . . $EEL_1L_1L_2L_2$	Pure	..
	S. I. 260, 261, 262, 267	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	489	184
	S. I. 264, 265, 268	Short . $EEL_1L_1l_2l_2$	...	Pure
1926	F_2 S. I. 157	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	144	33
1927	F_3 S. I. 299, 301	Long . . $EEL_1L_1L_2L_2$	Pure	..
	S. I. 300	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	81	27
	S. I. 302	Short . . $EEL_1L_1l_2l_2$	...	Pure
1926	F_2 S. I. 196	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	230	89
1927	F_3 S. I. 310	Long . . $EEL_1L_1L_2L_2$	Pure	...
	S. I. 309, 311	Long . . $EEL_1L_1L_2l_2$ (3 : 1)	80	27
	S. I. 312	Short . . $EEL_1L_1l_2l_2$	..	Pure

TABLE VII.

Segregating for 'long' and 'medium'. Pure for L_2 and segregating for E or L_1.

Season	Generation and family	Character of selection, constitution, and expected segregation	Bristle groups	
			Long	Medium
1927	F_2 S. I. 285	Long . . $EEL_1l_1L_2L_2$ or $EeL_1L_1L_2L_2$ (3 : 1)	18	6
	F_2 S. I. 524	Long . . Ditto .	96	33
	F_2 S. I. 838	Long . . Ditto .	271	73

TABLE VIII.

Segregating for 'medium' and 'short'. Segregating for E, L_1 or L_2.

Season	Generation and family	Character of selection, constitution, and expected segregation	Bristle groups	
			Medium	Short
1927	F_2 S. I. 569	Medium . . $Eel_1l_1L_2L_2$ $eeL_1l_1L_2L_2$ or $eeL_1L_1L_2l_2$ (3 : 1)	44	17
1928	F_2 S. I. 816	Medium . . Ditto .	81	24
1928	F_2 S. I. 842	Medium . . Ditto .	239	92
1929	F_2 S. I. 1068	Medium . . Ditto	242	76
1925	F_2 S. I. 128	Medium . . Ditto .	40	13
	F_3 S. I. 237, 239, 240, 241, 246, 247, 252, 253	Medium . . Ditto .	721	243
	S. I. 238, 242, 348, 254	Medium . . $EEl_1l_1L_2L_2$ or $eeL_1L_1L_2L_2$	Pure	...
1927	F_2 S. I. 290	Medium $Eel_1l_1L_2L_2$ $eeL_1l_1L_2L_2$ or $eeL_1L_1L_2l_2$ (3 : 1)	86	26

TABLE VIII—*contd.*

Season	Generation and family	Character of selection, constitution, and expected segregation	Bristle groups: Medium	Bristle groups: Short
1928	F_3 S. I. 877, 879, 880, 882, 883, 885, 886	Medium . . $EeI_1I_1L_2L_2$ $eeL_1I_1L_2L_2$ or $eeL_1L_1L_2I_2$ (3 : 1)	1723	568
	S. I. 881	Medium . . $EEI_1I_1L_2L_2$ or $eeL_1L_1L_2L_2$	Pure	...
	S. I. 878, 884	Short . . $eeL_1L_1I_2I_2$ or $eeI_1I_1L_2L_2$	...	Pure
1927	F_2 S. I. 293	Medium . . $EeI_1I_1L_2L_2$ $eeL_1I_1L_2L_2$ or $eeL_1L_1I_2I_2$ (3 : 1)	75	23
1928	F_3 S. I. 905	Medium . . Ditto	214	74
	S. I. 904, 906, 907	Medium . . $EEI_1I_1L_2L_2$ or $eeL_1L_1L_2L_2$	Pure	...
	S. I. 908, 909	Short . . $eeL_1L_1I_2I_2$ or $eeI_1I_1L_2L_2$	..	Pure

TABLE IX.

Segregating for 'short' and 'dwarf'. Segregating for E, L_1 or L_2.

Season	Generation and family	Character of selection, constitution, and expected segregation	Bristle groups: Short	Bristle groups: Dwarf
1928	F_2 S. I. 838	Short . . $EEL_1I_1I_2I_2$ $EeL_1I_1I_2I_2$ $eeL_1I_1I_2I_2$ or $eeI_1I_1L_2I_2$ (3 : 1)	161	37
1928	F_2 S. I. 1298	Short . . Ditto .	92	33
1928	F_2 S. I. 821	Short . . Ditto .	117	42
1929	F_3 S. I. 1116, 1117, 1118	Short . . Ditto .	624	196
	S. I. 1115	Short . . $EEL_1L_1I_2I_2$ $eeL_1L_1I_2I_2$ or $eeI_1I_1L_2L_2$	Pure	..

Segregations for the bristle characters have been met with concurrently with segregations for grain colour. Cross collations of bristle groups among grain colours have been made and point to no apparent connection between the factors determining bristle length and those responsible for grain colour groups. So also with segregates for anther colour.

Summary.

The bristles in *Setaria italica* fall into four groups—' long ', ' medium ', ' short ' and ' dwarf.' The ' dwarf ' bristle represents the basic bristle condition in all *Setarias*. This is due to a factor X. Three other factors E, L_1, and L_2 acting on X are responsible for the four differential lengths. E determines the expression of the various bristle types, and depends for its manifestation on the factor L_2. X with or without E remains a ' dwarf '. L_1 and L_2 contribute to the lengthening of the bristle. L_1 and L_2 acting individually on the dwarf (Xe) produce a short bristle ; together they produce a ' medium '. L_1 with XE gives a ' short '; L_2 with XE gives a ' medium '. L_1 and L_2 together with XE produce a ' long '.

The factors governing bristles and their expression are independent of those for grain (K, B and I) and anther colours.

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STUDIES IN INDIAN CHILLIES.

(3) THE INHERITANCE OF SOME CHARACTERS IN *CAPSICUM ANNUUM* L.*

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(With Plates XVI-XXI and eight text-figures).

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I. Introduction.

The unit species in this crop have already been described [Shaw and Abdur Rahman Khan, 1928] and a study of the root-systems [Ali Mohammad and Deshpande, 1929] has been made. The present paper deals with the inheritance of characters in a cross between two of the Pusa types. The work was undertaken with the object of investigating the genetical constitution of the types and also of providing a ready and practical demonstration of Mendelian theory for the training of post-graduate students. The work has afforded an admirable illustration for this latter purpose and it seems desirable to publish the results, not only for their value as an original research, but also as an example which may be of use to others who are engaged in educational work in plant genetics. As a secondary object it was hoped that the cross might yield some strains of economic significance. Apart from high yield, what is desired in a commercial type is that the fruits should be long,

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thin, red and pungent and should, at the same time, contain as little moisture as possible. The advantage with thin fruits, which generally possess very little moisture, is that the fruits dry up readily and retain a bright red colour, while thick fruits, which generally possess a high moisture per cent., take longer to dry and very often get mouldy and lose their attractive bright red colour and hence fetch a lower price.

Though red-fruited types are the principal types of commerce, it is said that in the United Provinces they prefer the yellow-fruited ones as they are believed to be more pungent than the red ones. However, both types of fruits are used, the yellow ones for their pungency, and the red ones for imparting an attractive red colour to the dishes.

The thick, non-pungent types, which are generally called " vegetable chillies ", cannot really be included under the commercial varieties. The demand for such varieties comes especially from big towns and cities and is very limited. They can neither be kept long without disposal nor can they stand transit well. Because of the high moisture percentage which they contain they are very susceptible to rotting.

No work has so far been published in India on the breeding or genetics of this crop, though this genus has been subjected to critical genetical analysis elsewhere by investigators such as Halsted [1911, 1914, 1915 and 1916], Webber [1912], Groth [1915], and Dale [1928, 1930] in U. S. A., Ikeno [1913, 1928] in Japan, and Atkins and Sherrad [1915] in England.

The results of the present cross generally agree with those of most other workers differing only in a few minor details. This suggests that the Indian types of chillies do not materially differ in their genetic constitution from those of other countries.

Investigating the inheritance of purple or violet colour in this plant, Ikeno obtained a 3 violet : 1 non-violet ratio in F_2, F_1 being a mosaic of purple and white or in other words intermediate for this character. He observed in F_2 four different grades of purple colour between the fully purple form of one parent and non-purple phenotype of the other parent; his paper, however, does not give the frequencies of these phenotypes. He has merely grouped the purple individuals together and found out their ratio to the non-purple individuals. His F_2 data, however, suggest a difference of more than one factor between the parents for this character. Had he carried his investigations to F_3 he would almost certainly have reached the conclusion that two factors are concerned in colour inheritance as we have done in this paper.

With regard to fruit-position, " pendent " or " erect ", Ikeno [1913], in his earlier investigations, got in F_1 an intermediate condition and obtained in F_2 a

1 pendent : 2 intermediate : 1 erect, a monohybrid ratio. He reports that in F_1 there was dominance of " erect " condition in the young fruit but when the fruits matured they drooped.

Ikeno's later investigations [1928], however, show that there was dominance of " erect " position in the F_1 in summer, while in the autumn the " pendent " position was dominant. The transition from one condition to the other was gradual so that on one and the same F_1 individual both the kinds of fruits were to be seen. Ikeno attributes this change of dominance to the difference in temperatures in the two seasons.

Dealing with the same character Webber obtained contrasting results in two different crosses. In one the " pendent " position, and in the other the " erect " was dominant. This indicates that the " fruit-position " in one of his two crosses was genetically different from that in the other, or in the light of Ikeno's results this difference in the behaviour of " fruit-position " in the two crosses may be the outcome of environmental influences. He also reports that there was variability in the F_1 of one of the crosses for this character which he attributes to impurity in one of the parents.

As regards the inheritance of colour of ripe fruit, the results of all previous workers—Webber, Ikeno, Atkins and Sherrad—suggest dominance of red colour over orange on a 3 : 1 basis.

Another character that gave conflicting results was the fruit-apex, pointed or blunt. Webber, in one cross, found F_1 to be intermediate for this character and in F_2 he got a 1 pointed : 2 intermediate : 1 blunt ratio, while in another cross the " pointed " condition was dominant.

Coming to quantitative characters, the only character, barring leaf-size [Webber, 1912], and dwarfishness of plant [Dale, 1930], which has so far drawn the attention of investigators is the " size of fruit." Ikeno, Webber and Groth obtained an intermediate F_1, and found in F_2 all forms ranging from that of the large parent on one side to that of the small parent on the other. Ikeno's results show a difference of four to five factors in the two parents for this character. Halsted did not fully realize the parental forms in F_2 even in a population of about one thousand plants. This suggests that the pod size was influenced by a large number of factors in this case.

The results of all these workers suggest that this size character in the pod of the chillies is due to additive or cumulative factors (of equal effect) giving an intermediate F_1 and a normal distribution in F_2.

Dale [1928], however, investigating the inheritance of length of fruit, got, instead of an intermediate F_1, dominance of short fruit, and obtained in F_2 a skew distribution in arithmetic classes instead of the normal distribution which would be

expected on the basis of cumulative factors. He explains this behaviour of fruit-length in his cross by saying that the factors involved for fruit-length in this case have proportionate rather than additive effects. This should give in F_2 a normal distribution in logarithmic classes, a result which was realized.

II. Technique of hybridization.

The technique of hybridization in this plant is easy. Although the size of the flowers is somewhat small, the flower structure is simple and no elaborate manipulations are necessary.

The flowers open in the morning, some time after sunrise, the majority opening between 8 and 10 A.M. The anthers commence dehiscing an hour or so after the opening of the flower. Both the opening of the flower and dehiscence of anthers are, to a large extent, dependent upon weather conditions. Shaw and Abdur Rahman Khan [1928], who have critically studied the biology of the flower, observed that on cold as well as on cloudy days the opening and dehiscence are delayed.

Emasculation.—This must be done before the flower opens. It may either be done early in the morning of the opening day of the flower or on the previous evening. The latter is always safer. All opened flowers should be removed from the mother plant. Buds which are due to open next morning swell up, and, in case of non-pigmented types, lose their greenish colour and appear white. With but a little experience one can easily know which of the buds will open the next day. Plump and healthy buds should be selected for the purpose of emasculation. With the help of a pair of forceps the petals are easily parted and anthers, five in number, removed. After emasculating a number of such buds on a plant small labels should be tied to them to distinguish them from unoperated ones. Better still is to slip over the buds on to the stalk a loop of some soft, coloured thread, made up of two to three strands. The advantage with this over the former is that this very easily yields to the growing fruit-stalk and the danger of the fruit-stalk being cut into by the thin thread of the label is eliminated. If there is no intention of doing any emasculation on the following one or two days, all unoperated buds which are likely to open on these days must be removed and the whole plant covered with a muslin bag. Bagging the entire plant is preferable to bagging individual flowers as this saves time and trouble in tying and untying several bags.

Pollination.—Late in the morning or early in the afternoon of the following day fresh flowers should be plucked from the intended male parent plant which has been previously bagged and pollen directly dusted on the stigmas of the emasculated flowers. The pollen is very dry and powdery and is easily dislodged from the anthers with a gentle shake. To facilitate pollination the petals of the pollen

flowers may be cut off. After pollination the bag should be replaced and labels denoting details should be tied outside the bag in the usual manner.

To avoid confusion and mistakes, one and the same plant should not be used as female parent for two or more different crosses.

Thin muslin bags have been found more satisfactory than paper bags as the setting under the latter is very low.

Even one successful pollination ensures enough seed supply to grow the F_1.

It has been found that at certain times of the season or during certain growth periods of the plant the percentage of setting is high while at others it is low or *nil*; it is therefore, important to select the right time for pollination. Crossing is generally found to be successful when the plant is in full bloom and natural setting high. Pollinations should be avoided, as far as possible, during cloudy weather as setting is very low in such a weather.

Selfing.—Thin muslin bags of the size 3 ft. 6 in. × 1 ft. 2 in. have been found very satisfactory for selfing a plant. Before bagging a plant all fruits and open flowers must be plucked. Whether the plant to be selfed is from a hybrid population or from a pure line, it should never be entirely covered if any characters are to be studied, but a branch should always be left out for observations as this saves the bother of untying and tying it every time a character is to be observed beside reducing the chances of accidental cross-pollination to the minimum.

It was found necessary to shake the bags at noon every day as this dislodges the pollen from the anthers and helps in pollination. If this is not done the setting is found to be very poor.

III. Description of parents and F_1.

For the purpose of this research two types which showed very sharply contrasting characters were chosen. The following is the description of the parents [Shaw and Abdur Rahman Khan, 1928].

Type 3.—Plants tall (66·0 cm.), intermediate in maturity, poor in bearing, annual; leaf 8·5 cm. × 4·2 cm., purple; flower one in the axil; pedicel nearly equal to the fruit; corolla purple; calyx not enclosing the base of the fruit; style purple; stigma purple; fruit short, globular, 1·2 cm. × 1·4 cm., 3-celled, circular, in transverse section, erect, unripe purple becoming red when mature, apex blunt, flesh thick, average weight of a fresh ripe fruit 0·98 gram.

Type 29.—Plants very tall (83·0 cm.), late, prolific, annual; leaf 13·0 cm. × 6·0 cm., dark green; flower one in the axil; pedicel shorter than the fruit; corolla white; calyx enclosing the base of the fruit; style purple; fruit long, 12·0 cm. × 1·0 cm., 2-celled, elongated, circular in transverse section, pendent, unripe light green with moderate purple colour, becoming orange when mature, apex acute flesh thin, average weight of a fresh ripe fruit 5·5 grams.

We found that purple colour in the style of Type 29 was very variable—some flowers showing almost white styles.

The characters of the parents and the F_1 are shown below (Tables I and II). It will be seen that the F_1 is intermediate in most respects but that red colour in the fruit is dominant to orange, the pendent position dominant to the erect, and "calyx not enclosing fruit base" dominant to "enclosing fruit base".

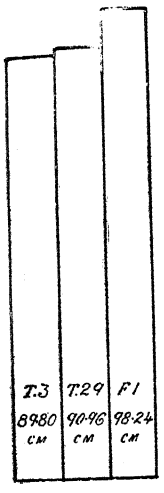
In quantitative characters the F_1 was intermediate for lengths of petal, fruit and fruit-stalk. The breadth of petal was less than that in either parent; short pedicel was dominant to long pedicel; and greater weight of fruit (dry and undry) was dominant to lesser weight. In the following characters, however, the F_1 showed heterosis:—general vigour, maturity, height of plant, productivity (both in the total number of fruits produced and in the total weight of dry produce) and thickness of fruit. Some of the phenomena have been illustrated graphically and with the help of a photograph (Plate XVI, figs. 1 and 2).

The reciprocal cross, Type 29 × Type 3, having failed, had to be repeated the following year. The F_1, F_2 and F_3 results are in close agreement with those of the original cross, Type 3 × Type 29.

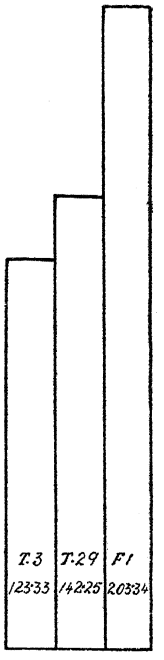
TABLE I.

Description of the qualitative characters of the parents and F_1.

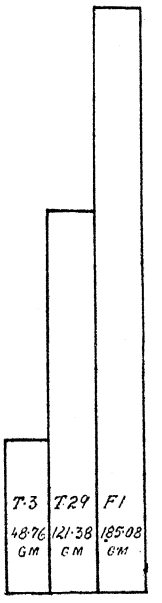
Family	Shoot			
	Foliage colour in mass	Lamina colour	Petiole colour	Stem colour
Type 3 parent	Dark purplish green	Young—dark green; margin apex, veins purplish. Old—upper surface dark purple; lower dark green; veins light purple	Purplish .	Woody portion with grey streaks; herbaceous, dark purple
Type 29 parent	Green . .	Green . . .	Green . .	Woody portion grey; herbaceous, green; upper surface of very young branches light purple; nodes purple
Type 3 × Type 29 and Type 29 × Type 3, F_1	Very dark green	Dark green with a slight purple tinge; margin and marginal veins purplish	Green; upper surface purplish	Like Type 29 but purple colour more deep and young branches purplish all over
Remarks about F_1	Intermediate	Intermediate	Intermediate	Intermediate



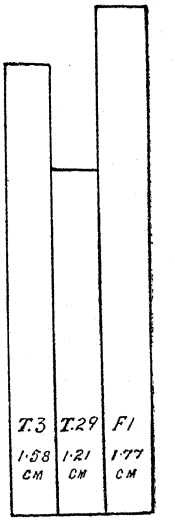
Height of plant.



Total number of fruits per plant.

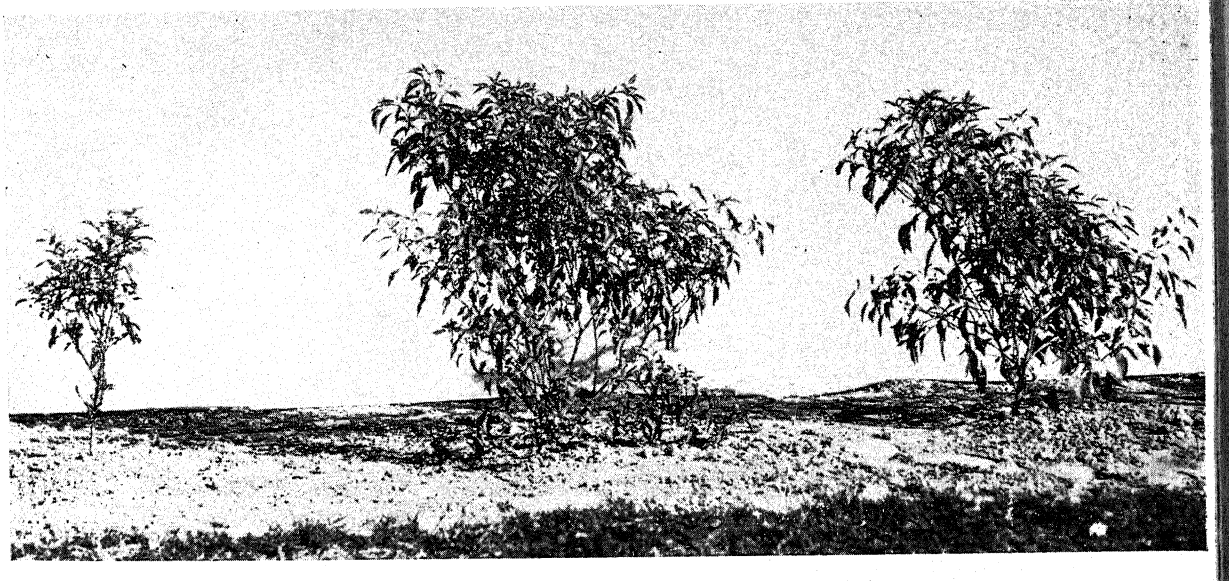


Total dry produce per plant.



Fruit—thickness.

Fig. 1.—Heterosis in F_1.



T. 3. F_1. T. 29.

Fig. 2.—Heterosis in F_1. Vigour and height of plant.

TABLE I—*contd.*

Description of the qualitative characters of the parents and F_1—contd.

Family	FLOWER					
	Calyx and pedicel colour	Corolla colour	Anther colour	Filament colour	Style colour	Stigma colour
Type 3 parent	Greenish purple	Purple or white splashed with purple; margin and apex of petal purple	Bluish purple	Purple .	Purple .	Purple
Type 29 parent	Calyx green; pedicel purplish at base	White . .	Yellow at base; purplish towards apex	White .	Variable from light purple to white	Yellow
Type 3 × Type 29 and Type 29 × Type 3, F_1	Purplish green	White with a light purple apex and margins; colour sometimes extending inwards	Light bluish purple	Light purple; nearly white at base	Light purple	Light purple
Remarks about F_1	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate

Family	FRUIT							
	Fruit colour		Position	Shape	Calyx	Apex	Base	Seed colour
	Unripe	Ripe						
Type 3 parent	Purple	Dark red	Erect	Globose	Not enclosing fruit-base	Blunt	Bulged	Reddish yellow
Type 29 parent	Green, exposed parts purplish	Bright orange	Pendent	Elongate	Enclosing fruit-base	Pointed	Non-bulged	Yellow
Type 3 × Type 29 and Type 29 × Type 3, F_1	Light purple	Red	Pendent	Elongate and globose at base	Not enclosing fruit-base	Partially pointed	Bulged	Reddish yellow
Remarks about F_1	Intermediate	Red dominant to orange	Pendent dominant to erect	Intermediate	"Not enclosing" dominant to "enclosing"	Intermediate	Bulged dominant to non-bulged	Intermediate

TABLE II.

Description of the quantitative characters of the parents and the F_1.

Character	Type 3 parent, mean	Type 29 parent, mean	F_1		Remarks about F_1
			Type 3 × Type 29, mean	Type 29 × Type 3, mean	
Length of petal in mm.	9·46	15·38	11·05	11·22	Intermediate
Breadth of petal in mm.	6·49	7·04	5·76	5·81	Less broad than either parent
Length of pedicel in mm.	11·22	24·29	12·40	11·59	Like the short pedicelled parent, *i.e.*, short pedicel dominant to long
Length of fruit in cm.	1·28	11·05	5·30	5·44	Intermediate
Thickness of fruit in cm.	1·56	1·21	1·78	1·80	Thicker than either parent
Length of fruit-stalk in cm.	2·06	4·13	3·10	3·05	Intermediate
Height of plant in cm.	89·80	90·96	98·24	100·50	Taller than either parent
Weight of ripe, undried fruit in grms.	1·67	5·32	4·50	4·72	Somewhat like heavy fruited parent
Weight of ripe, dried fruit in grm.	0·40	0·86	0·91	0·93	" "
Total number of fruits per plant	121·90	141·02	203·34	207·75	More prolific than either parent
Total dry produce per plant in grms.	48·76	121·38	185·08	189·45	Greater than that in either parent

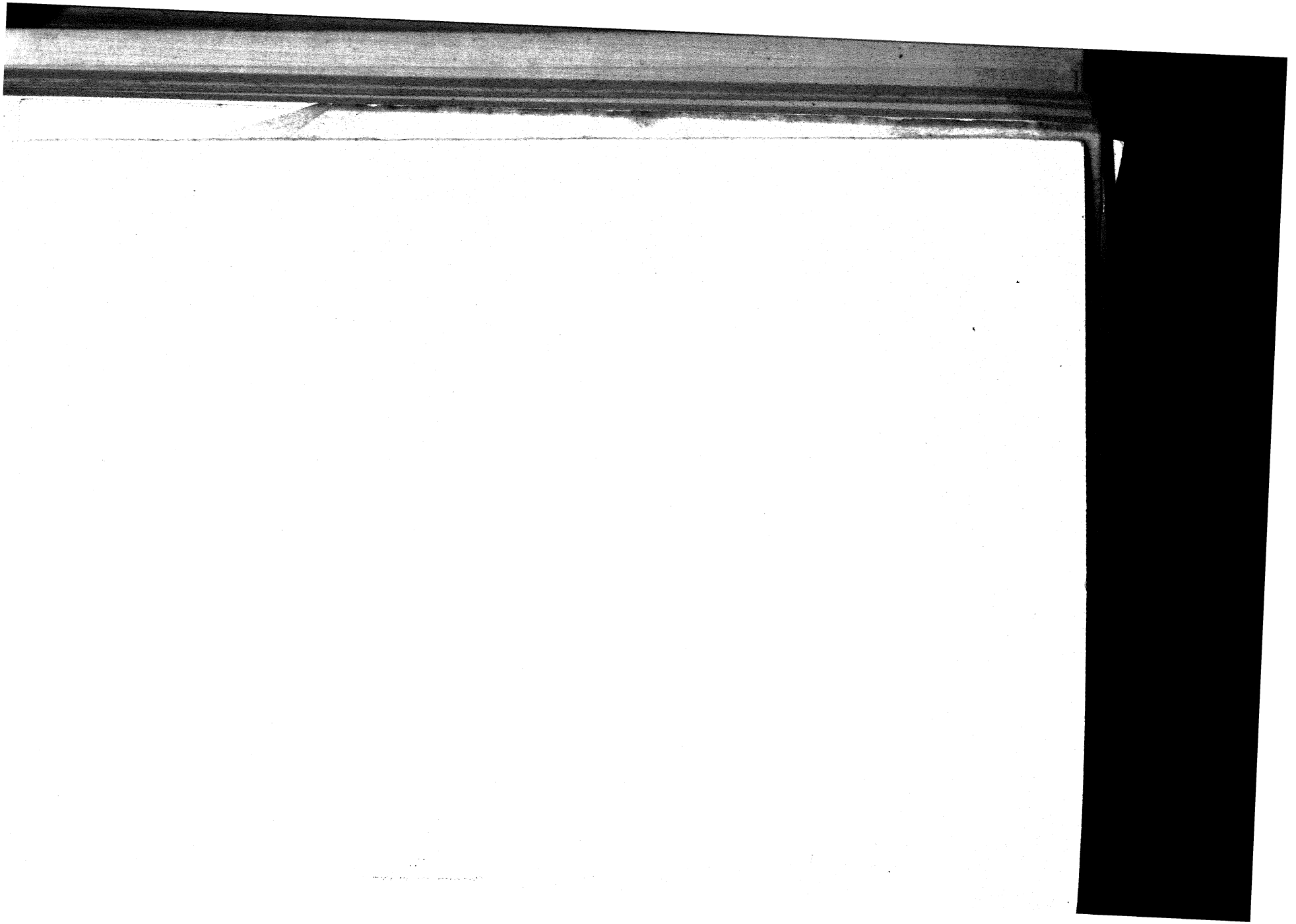
IV. THE F_1 GENERATION.

The hybrid seeds of the cross Type 3 × Type 29 were sown in dealwood boxes in the 1st week of August, 1928, and those of the reciprocal in the following year about the same time along with the seeds of the parents. The seeds germinated after about five days. Even at this early stage the cotyledonary leaves and the hypocotyl showed the F_1 to be intermediate for purple colour.

Within a short time after germination the F_1 seedlings showed more rapid growth than the parental seedlings and at the time of transplanting were nearly one and a half times taller than the latter. Not only were they taller than the parental seedlings but were also much more vigorous.

The seedlings were transplanted in the field in the beginning of September, at a distance of two-and-a-half feet between plants and between rows. The two parents were grown on either side of the F_1. Thirty plants were grown of the cross Type 3 × Type 29 and four of the reciprocal.

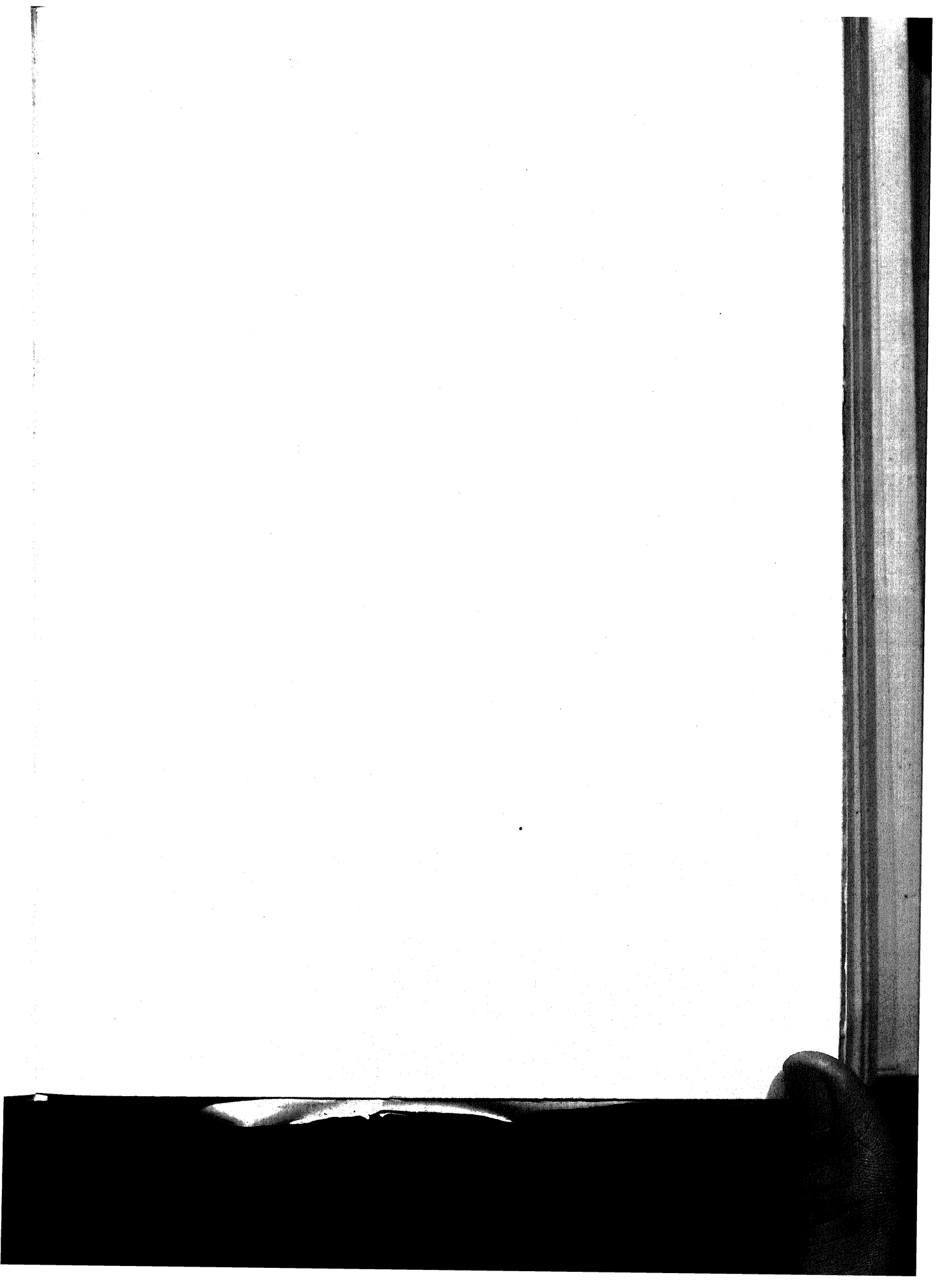
The earlier observations as to the intermediacy of purple colour in the F_1 were confirmed subsequently. The F plants were the first to flower and were earlier by



T. 29.

F_1.
COLOUR IN PLANT.

T. 3.



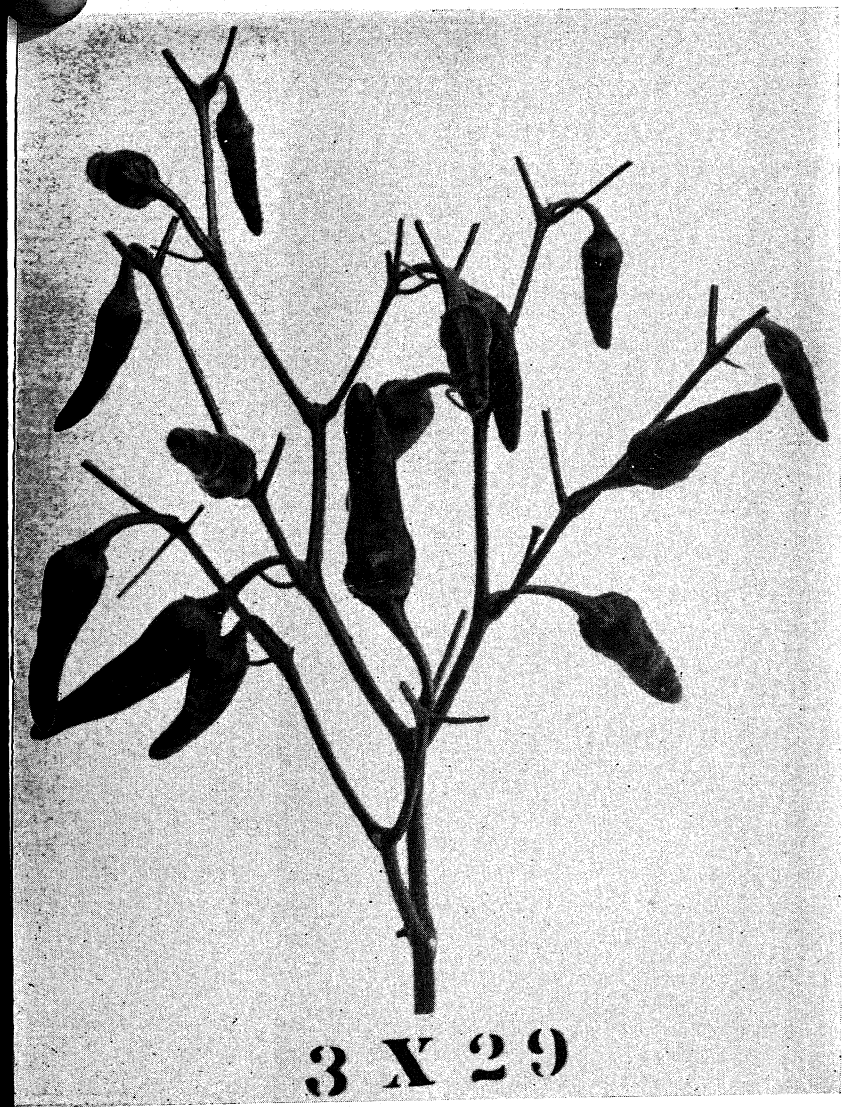


Fig. 1.—Close view.

Fig. 2.—Distant view.

Variation in fruit-position in F_1.

about a week than the earlier parent. The flowers were whitish with coloured petal margins, apices and central veins (Plate XVII). The colour extended also inwards a little, depending upon the illumination which a flower received.

The next qualitative character that was studied in the F_1 plants was the "fruit-position". It proved to be very puzzling at the start as in the majority of F_1 plants the few earliest fruits were either erect, intermediate (horizontal) or both. While in a few others all the fruits, including the earliest, were only pendent. Thus it was difficult to say, just at the beginning, as to which of the positions—pendent or erect—was dominant or whether this character was intermediate, like the colour character. The real state of affairs was revealed when all the subsequent fruits in all the plants were found to be pendent, suggesting that the pendent condition is dominant over the erect condition. Table III, given below, shows the behaviour of all the F_1 plants regarding the position of their fruits, and the photographs of two F_1 plants (Plate XVIII, figs. 1 and 2) illustrate all the three different fruit-positions—erect, intermediate and pendent—in one and the same individual.

TABLE III.

Behaviour of F_1 plants for fruit-position.

Plant No.	No. of erect fruits	No. of intermediate fruits	No. of pendent fruits
	Type 3 × Type 29		
1	1	2	153
2	5	0	178
3*	0	0	178
4	0	0	431
5*	0	1	423
6	1	0	154
7	8	0	218
8*	3	0	265
9	0	2	383
10*	0	0	120
11	0	0	169
12	4	4	249
13*	2	0	202
14	0	0	269
15*	2	0	171
16	0	0	57
17	2	0	166
18*	0	0	146
19	0	3	230
20*	3	0	149
21	Died before flowering		
22	1	0	142
23*	1	0	140

* Plants selected for F_2

TABLE III—*contd.*

Plant No.	No. of erect fruits	No. of intermediate fruits	No. of pendent fruits
24	2	2	208
25*	3	0	128
26	0	1	88
27	3	0	97
28*	0	0	119
29	3	0	440
30*	2	0	163
	Type 29 × Type 3		
1*	0	0	231
2*	2	0	198
3*	0	0	214
4*	0	0	98

* Plants selected for F_2

The F_1 fruits, on ripening, were red proving the dominance of this colour over orange. The red colour, however, looked lighter due to the fact that the F_1 was less purple than Type 3 (Plate XIX).

It was decided to self twelve F_1 plants of the cross Type 3×Type 29 and all the four of the reciprocal to grow the F_2 generation and so every third and fifth plant in the line was bagged in the former case and all the four in the latter. Setting was very good in each case and sufficient seed to grow a large F_2 progeny was obtained.

V. THE F_2 AND F_3 GENERATIONS.

The seeds of the twelve selected F_1 plants of Type 3×Type 29 were sown in dealwood boxes, separately, in the first week of August, 1929, and those of the four of the reciprocal the following year, and seedlings were transplanted in the field by the end of August of the respective years. In uprooting the seedlings for transplanting care was taken to remove them starting from one side of the seed-bed to avoid selection as the coolies who are employed to do this work are apt to be partial to coloured or taller seedlings if this method is not insisted upon.

One row, of ninety-six plants each, was given to the progeny of each of the twelve F_1 plants of Type 3 × Type 29 thus making up the entire F_2 population to number 1152. The object of growing such a large F_2 population was obviously to enable realization of the " recessive " in qualitative characters and parental forms in quantitative characters if any of them were to be influenced by several factors.

Non-purple (like T. 29)

Purple grade I (like F_1)

Purple grade II (intermediate between F_1 and T. 3)

Purple grade III (like T. 3)

F_2 PHENOTYPES FOR COLOUR IN PLANT.

The parents were also grown by the side of the F_2. A line of 85 plants was given to each of the four F_1 plants of the reciprocal.

A few plants died some time after transplantation due to attacks of white ants and other insects, but care was taken to replace them with plants of the same colour as far as possible.

In all seventy-four plants, representing all possible combinations, were selected as parents of the F_3 cultures of Type 3 × Type 29 and 54 of the reciprocal. They were bagged and F_3 was grown from selfed seed except in those few cases where bagged seed was not obtained at all or was insufficient.

The bagged plants were occasionally found infested with aphis and had to be exposed to the sun. During the time these were uncovered, care was taken to remove all flowers opening each day. Also as a precaution against mistakes a tag was tied to the base of a bagged branch or branches, to distinguish them from others not bagged in case of all those plants thus exposed.

For the study of quantitative characters five measurements were taken on one plant for each character in the parents, F_1, F_2 and F_3, selecting as far as possible variates to represent the average condition. Quantitative characters were not studied in F_2 and F_3 of the reciprocal, the population being small.

In measuring lengths of fruits and fruit-stalks use of a stiff piece of twine was made as this enabled us to measure curved variates with accuracy and without much difficulty. Fruit was measured from its apex to the point of its attachment to the stalk, and the fruit-stalk from the point of its attachment to the calyx to its base.

Thickness of fruit was measured at the thickest place with a pair of calipers.

Length and breadth of petal were measured by keeping the petal flat on a graph-paper. Help of a smooth, flat petri-dish cover was taken to keep the petal flat and well spread.

Purple colour.

Type 3 parent has purple colour in all its vegetative and reproductive organs while Type 29 parent lacks it. The F_1 was intermediate for this character and the F_2 population showed the following phenotypes :—

(1) Purple grade * III (like Type 3), (2) Purple grade II (less purple than (1)), (3) Purple grade I, like F_1 ; and Non-purple, like Type 29 (Plate XX).

* The abbreviations Pr. and gr. have been used for Purple and grade respectively at places.

The F_2 frequencies for each of the twelve F_1 plants of Type 3 × Type 29 were as shown below :—

Type 3 × Type 29—F_2.

F_1 plant No.	Purple			Non-purple	Total
	gr. III	gr. II	gr. I		
3	5	14	48	28	95
5	6	21	38	26	91
8	5	18	43	25	91
10	4	14	50	28	96
13	3	14	51	22	90
15	3	16	51	18	88
18	7	22	47	16	92
20	9	15	48	19	91
23	9	19	50	18	96
25	2	22	41	6	85
28	10	14	49	20	93
30	2	14	47	29	92
Total observed .	65	203	563	269	1,100
Total expected on 1 : 3 : 8 : 4 basis	68·75	206·25	550	275	1,100
Ratio observed .	0·94 :	2·95 :	8·19 :	3·91	
Ratio expected .	1 :	3 :	8 :	4	

$\chi^2 = 0{\cdot}693$, *i.e.*, less than one, hence the fit is excellent.

Combining all purples together and taking them against non-purples the following frequencies are obtained :—

	Purples	Non-purples	
Total observed	831	269	=1,100
Total expected on 3 : 1 basis . .	825	275	=1,100
Ratio observed	3·02 :	0·98	
Ratio expected	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{6}{9{\cdot}62} = 0{\cdot}62.$$

The fit, therefore, is excellent.

In the case of some families the observed frequencies do not show a very close agreement with theory ; as, however, the total frequency within a family is generally about 90 and the segregation is on a dihybrid ratio, this is only to be expected. The fit for the combined frequencies of all the families is good and as is shown below, the results of F_3 confirm the F_2 theory.

The following segregations occurred in the seventy-four cultures in F_3 :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
4 cultures from purple grade III (like Type 3).	Pure	AABB .	4	4
16 cultures from purple grade II (intermediate between purple grade III and grade I).	Pure	AAbb .	6	5·3
	Pr. gr. II Pr. gr. III 3 : 1 . .	AABb .	10	10·6
31 cultures from purple grade I (like F_1).	Like F_2	AaBb .	13	15·5
	Pr. gr. III Pr. gr. I Non-purple 1 : 2 : 1	AaBB .	10	7·75
	Pr. gr. II Pr. gr. I : Non-purple 1 : 2 : 1	Aabb .	8	7·75
23 non-purple cultures . .	All non-purple . . .	aaBB .	23	23
		aaBb .		
		aabb .		

Type 29 × Type 3—F_2.

F_1 plant No.	Purple			Non-purple	Total
	gr. III	gr. II	gr. I		
1	4	13	46	23	86
2	1	13	42	29	85
3	8	17	41	18	84
4	6	13	48	17	84
Total observed .	19	56	177	87	339
Total expected on 1 : 3 : 8 : 4 basis	21·19	63·57	169·50	84·75	339·01
Ratio observed .	0·89 :	2·64 :	8·35 :	4·10	
Ratio expected .	1 :	3 :	8 :	4	

$\chi^2 = 1{\cdot}62$.

$P = 0{\cdot}68$. **Therefore, the fit is good.**

Combining all purples together and taking them against non-purples the following frequencies are obtained :—

	Purples	Non-purples	
Total observed	252	87	=339
Total expected on 3 : 1 basis . .	254·25	84·75	=339
Ratio observed	2·97 :	1·03	
Ratio expected	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{2{\cdot}25}{5{\cdot}34} = 0{\cdot}42.$$

Therefore, the fit is good,

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
2 cultures from purple grade III (like Type 3)	Pure	AABB .	2	2
15 cultures from purple grade II (intermediate between purple grade III and grade I)	Pure	AAbb .	4	5
	Pr. gr. II Pr. gr. III 3 : 1	AABb .	11	10
23 cultures from purple grade I (like F_1)	Like F_2	AaBb .	12	11·50
	Pr. gr. III Pr. gr. I Non-purple 1 : 2 : 1	AaBB .	4	5·75
	Pr. gr. II Pr. gr. I Non-purple 1 : 2 : 1	Aabb .	7	5·75
18 non-purple cultures . .	All non-purple . . .	aaBB .	18	18
		aaBb .		
		aabb .		

The behaviour of F_3, therefore, confirms the theory that the cross shows dihybrid segregation for colour in plant.

Fruit-position.

Type 3 has erect fruits while the fruits of Type 29 are pendent. The F_1 had pendent fruits excepting the earliest fruits in the majority of the plants which were either erect or intermediate. Reference to this has already been made in describing the F_1 (page 227, Plate XVIII).

The F_2 population showed the following phenotypes and frequencies :—

Type 3 × Type 29—F_2.

F_1 plant No.	Pendent-fruited plants	Erect-fruited plants	Total
3	69	26	95
5	63	27	90
8	70	21	91
10	74	22	96
13	68	20	88
15	61	26	87
18	69	23	92
20	68	23	91
23	78	18	96
25	65	20	85
28	70	23	93
30	69	23	92
Total observed .	824	272	1,096
Total expected on 3 : 1 basis .	822	274	1,096
Ratio observed .	3·01 :	0·99	
Ratio expected .	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{2}{9{\cdot}66} = 0{\cdot}207$$

The fit, therefore, is excellent.

Some of the F_2 plants had, as in F_1, their early fruits either erect or intermediate or both, the subsequent fruits, however, remaining pendent.

These F_2 plants were not necessarily from those F_1 plants which had showed similar behaviour as will be seen from Table IV.

TABLE IV.

F_1 plant No. (T. 3 × T. 29)	F_1 condition	F_2 condition	F_2 plant No.
3	All pendent	Intermediate and pendent . .	23
5	Intermediate and pendent . .	„ „ . .	69, 72
8	Erect and pendent . . .	Erect and pendent . . .	70
13	„ „ . . .	„ „ . . .	42
15	„ „ . . .	„ „ . . .	54
15	„ „ . . .	Erect, intermediate, pendent .	81
18	All pendent	Erect and pendent . . .	4, 6
20	Erect and pendent . . .	Intermediate and pendent . .	75
28	All pendent	Erect and pendent . . .	13
30	Erect and pendent . . .	„ „ . . .	7, 69

Even though the F_1 plant Nos. 23 and 25 of Type 3 × Type 29 and No. 2 of the reciprocal had, in F_1, their earliest fruits erect (1 in No. 23 and 3 in No. 25 in the former case and 2 in No. 2 in the latter), in F_2, both had only pendent fruits. This indicates that there is absolutely no correlation between one generation and the next as regards this aberrant behaviour in the position of fruits.

Out of the seventy-four F_3 cultures, fifty were from pendent-fruited plants and twenty-four from erect-fruited plants. Their behaviour in F_3 was as follows :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
50 cultures from pendent-fruited plants	Pure	PP . .	10	16·67
	Pendent Erect 3 : 1 . . .	Pp . .	40	33·34
24 cultures from erect-fruited plants	Pure	pp . .	24	24

Type 29 × Type 3—F_2.

F_1 plant No.	Pendent-fruited plants	Erect-fruited plants	Total
1	62	19	81
2	57	21	78
3	60	22	82
4	65	13	78
Total observed	244	75	319
Total expected on 3 : 1 basis	239·25	79·75	319
Ratio observed	3·06 :	0·94	
Ratio expected	3 :	1	

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{4·75}{5·18} = 0·91.$

The fit is good.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
45 cultures from pendent-fruited plants	Pure	PP	19	15
	Pendent : Erect 3 : 1	Pp	26	30
13 cultures from erect-fruited plants	Pure	pp	13	13

The F_3 results agree fairly well with the monohybrid segregation on a 3 : 1 basis in F_2 though the deviation from the expected numbers in the case of the behaviour of pendent-fruited cultures of Type 3 × Type 29 is rather high. However, the back-cross further confirms the above results (page 286).

Colour of ripe fruit.

The ripe fruits of Type 3 parent are red and the purple colour in the plant gives them a dull or darkish appearance. Type 29 has bright orange ripe fruits. The F_1 was red-fruited with a less dull tinge (Plate XIX).

The F_2 population showed the following phenotypes and frequencies :—

Type 3 × Type 29—F_2.

F_1 plant No.	Red-fruited plants	Orange-fruited plants	Total
3	70	23	93
5	70	19	89
8	67	23	90
10	73	22	95
13	73	15	88
15	69	17	86
18	69	23	92
20	62	26	88
23	74	21	95
25	68	16	84
28	67	25	92
30	63	27	90
Total observed	825	257	1,082
Total expected on 3 : 1 basis	811·5	270·5	1,082
Ratio observed	3·05 :	0·95	
Ratio expected	3 :	1	

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{13{\cdot}5}{9{\cdot}60} = 1{\cdot}40.$

The fit is, therefore, good.

The behaviour of red- and orange-fruited F_2 plants in F_3 was as follows :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
56 cultures from red-fruited plants	Pure	RR	16	18·67
	Red Orange 3 : 1	Rr	40	37·34
18 cultures from orange-fruited plants	Pure	rr	18	18

Type 29 × Type 3—F_2.

F_1 plant No.	Red-fruited plants	Orange-fruited plants	Total
1	69	11	80
2	54	23	77
3	62	20	82
4	52	23	75
Total observed	237	77	314
Total expected on 3 : 1 basis	235·5	78·5	314
Ratio observed	3·02 :	0·98	
Ratio expected	3 :	1	

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{1·5}{5·14} = 0·29.$

The fit is good.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
45 cultures from red-fruited plants	Pure	RR	13	15
	Red Orange 3 : 1	Rr	32	30
13 cultures from orange-fruited plants	Pure	rr	13	13

The F_3 results, therefore, confirm the F_2 segregation on a monohybrid basis. This is further confirmed by the back-cross (page 288).

Fruit-apex.

The fruit of Type 3 has a blunt apex while that of Type 29 has pointed. The F_1 fruit was partially pointed, or, in other words, it was intermediate for this character. In F_2 the following phenotypes and frequencies were observed.

Type 3 × Type 29—F_2.

F_1 plant No.	Pointed and partially pointed apex	Blunt apex	Total
3	70	18	88
5	67	18	85
8	60	27	87
10	75	17	92
13	67	20	87
15	63	22	85
18	61	29	90
20	61	20	81
23	67	26	93
25	56	25	81
28	69	23	92
30	78	14	92
Total observed	794	259	1,053
Total expected on 3 : 1 basis	789·75	263·25	1,053
Ratio observed	3·02 :	0·98	
Ratio expected	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{4{\cdot}25}{9{\cdot}47} = 0{\cdot}44.$$

The fit, therefore, is excellent.

As it was found difficult to classify the fruits into "pointed" and "partially pointed" classes due to variation on the same plant, the fruits belonging to both these classes have been included in one group. Because of this reason in F_3 a few cultures with typically "pointed" and typically "partially pointed" apices were only observed and by chance all the former proved to be homozygous and all the latter heterozygous as would be expected on theory. The results of F_3 observations are shown below.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
9 cultures from plants with pointed fruits	Pure	dd	9	9
14 cultures from plants with partially pointed fruits	Like F_2 . . .	Dd	14	14
13 cultures from plants with blunt fruits	Pure	DD	12	13

Type 29 × Type 3—F_2.

F_1 plant No.	Pointed and partially pointed apex	Blunt apex	Total
1	58	22	80
2	58	23	81
3	63	17	80
4	61	16	77
Total observed .	240	78	318
Total expected on 3 : 1 basis .	238·5	79·5	318
Ratio observed .	3·02 :	0·98	
Ratio expected .	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{1{\cdot}5}{5{\cdot}21} = 0{\cdot}29.$$

The fit is excellent.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
7 cultures from plants with pointed fruits	Pure	dd	7	7
12 cultures from plants with partially pointed fruits	Like F_2 . . .	Dd	12	12
9 cultures from plants with blunt fruits	Pure	DD	9	9

The F_3 results confirm the monohybrid segregation in F_2.

All the 13 cultures from plants with blunt fruits in Type 3 × Type 29 should be pure on theory but one was found to segregate like F_2. The F_2 diagnosis in this case seems to be wrong.

Fruit-base.

The fruit-base of Type 3 is roundish or bulged while that of Type 29 is merely cylindrical or not bulged at base. The F_1 plants segregated for this character as follows :—

Type 3×Type 29—F_2.

F_1 plant No.	Bulged	Not-bulged	Total
3	63	26	89
5	66	19	85
8	72	17	89
10	69	24	93
13	66	21	87
15	64	19	83
18	76	14	90
20	59	23	82
23	80	15	95
25	64	19	83
28	77	13	90
30	63	27	90
Total observed .	819	237	1,056
Total expected on 3 : 1 basis .	792	264	1,056
Ratio observed .	3·11 :	0·89	
Ratio expected .	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{27}{9·49} = 2·84.$$

The fit, therefore, is fairly good.

There were nine doubtful cases which have not been taken into account.

The following segregations occurred in F_3 :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
6 cultures from plants with fruit-base "not bulged"	Pure	ff	6	6
29 cultures from plants with fruit-base "bulged"	Pure	FF	11	9·66
	Like F_2	Ff	18	19·32

Type 29×Type 3—F_2.

F_1 plant No.	Bulged	Not-bulged	Total
1	57	23	80
2	57	24	81
3	58	22	80
4	56	21	77
Total observed	228	90	318
Total expected on 3 : 1 basis	238·5	79·5	318
Ratio observed	2·87 :	1·13	
Ratio expected	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{10{\cdot}5}{5{\cdot}21} = 2{\cdot}02.$$

The fit is good.

Type 29×Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
7 cultures from plants with fruit-base "not bulged"	Pure	ff	7	7
21 cultures from plants with fruit-base "bulged"	Pure	FF	5	7
	Like F_2	Ff	16	14

The observations in F_3 confirm the F_2 results.

Calyx behaviour," enclosing fruit-base" or "not enclosing fruit-base".

The base of the fruit of Type 3 is not enclosed by the calyx while that of Type 29 is enclosed (like the finger-tip by a thimble). Calyx of the F_1 fruit seemed to enclose the base in the earlier stages but later due to bulging of base of fruit it

remained unenclosed. The F_1 plants showed the following phenotypes and frequencies in F_2 :—

Type 3×Type 29—F_2.

F_1 plant No.	Fruit-base not enclosed	Fruit-base enclosed	Total
3	64	27	91
5	67	19	86
8	72	18	90
10	72	22	94
13	65	18	83
15	67	19	86
18	72	17	89
20	63	17	80
23	82	12	94
25	68	17	85
28	79	13	92
30	62	28	90
Total observed	833	227	1,060
Total expected on 3 : 1 basis	795	265	1,060
Ratio observed	3·15 :	0·85	
Ratio expected	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{38}{9{\cdot}51} = 3{\cdot}99.$$

The fit, therefore, is bad. There were eleven doubtful cases which have been omitted. If they all be added to the class in which there is deficiency, the fit improves as shown below. The deviation becomes 29·75 and probable error 9·5.

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{29{\cdot}75}{9{\cdot}51} = 3{\cdot}13.$$

The following segregations were observed in F_3 :—

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
29 cultures from plants with fruit-base "not enclosed"	Pure	EE	12	9·66
	Like F_2	Ee	17	19·32
6 cultures from plants with fruit-base "enclosed"	Pure	ee	6	6

Type 29×Type 3—F_2.

F_1 plant No.	Fruit-base not enclosed	Fruit-base enclosed	Total
1	57	23	80
2	57	24	81
3	58	22	80
4	56	21	77
Total observed .	228	90	318
Total expected on 3 : 1 basis .	238·5	79·5	318
Ratio observed .	2·87 :	1·13	
Ratio expected .	3 :	1	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{10{\cdot}5}{5{\cdot}21} = 2{\cdot}02.$$

The fit is good.

Type 29×Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
21 cultures from plants with fruit-base "not enclosed"	Pure	EE	5	7
	Like F_2	Ee	16	14
7 cultures from plants with fruit-base "enclosed"	Pure	ee	7	7

The F_3 observations confirm the F_2 results.

Colour in style, stigma, anther and unripe fruit.

The colour in these organs was found to be completely linked with the vegetative colour. Whenever there was purple colour present in the vegetative part of a plant these organs were purple. When this purple colour was absent the style remained light purple or white *, stigma yellow, anther bluish-yellow, and unripe fruit green, except a light purple patch on the exposed side.

Seed-colour.

The seed-colour was linked with the colour of fruit. Red fruits had reddish-yellow seeds while orange or yellow fruits had yellowish seeds (Plate XIX).

The number of locules in the fruit.

The number of locules in Type 3 fruit varies from two to three, while in Type 29 the number is always two. The F_1 had two-loculed fruits. In F_2 the

* The study of the inheritance of the purple colour in the style of Type 29 was rendered difficult due to the variation in colour (from coloured to white) in the same plant and hence had to be abandoned. It should be remembered that this purple colour in the style of Type 29, which is light, is quite distinct from that present in Type 3.

number varied from two to even five in the same plant although the thin fruits were invariably two-loculed. Because of this variability this character was not further studied.

Fruit-shape.

Type 3 has globose fruits and Type 29 elongate. F_1 had elongate fruits but nearly globose at base, that is, F_1 had partly taken both the characters, one from each parent (Plates XIX and XXI).

In F_2, in a population of over a thousand, only six plants had round or roundish fruits but they were not in any way as round as the fruits of Type 3 (Plate XXI). In F_3 all the above cultures segregated with all shapes from perfectly round to short and conical fruits. This suggests that the Type 3 parental shape was not realized in F_2 and that this character is influenced by a large number of factors.

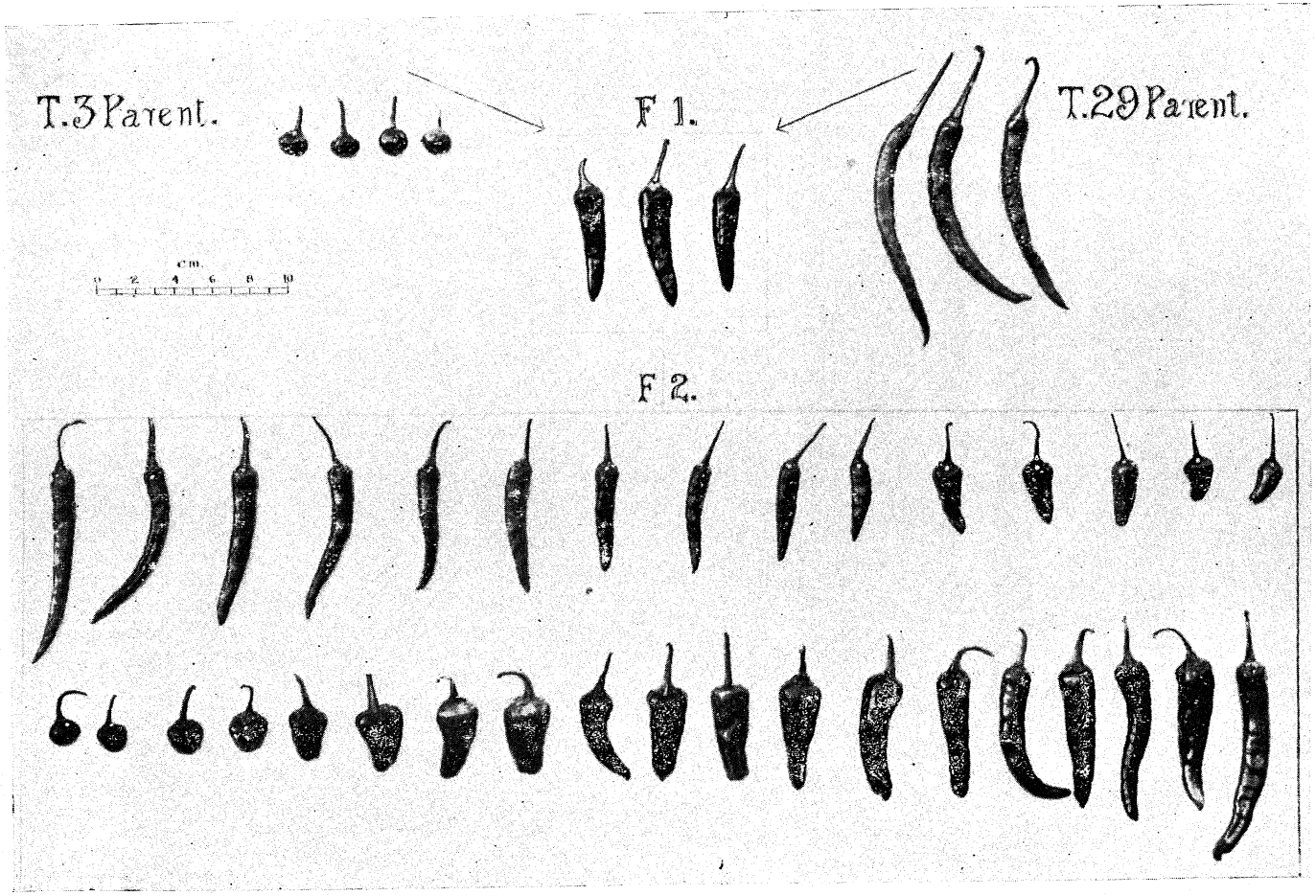
Each of the foregoing characters has been considered independently so far but now an attempt has been made to show the behaviour in the case of certain qualitative characters, *viz.*, purple colour, fruit-position and colour of ripe fruit, when any two of them or all the three are considered together as follows :—

Purple colour in plant and fruit-position together.

Type 3×Type 29—F_2.

F_1 plant No.	Purple						Non-purple		Total
	Gr. III pendent	Gr. III erect	Gr. II pendent	Gr. II erect	Gr. I pendent	Gr. I erect	Pendent	Erect	
3	5	0	12	2	29	19	23	5	95
5	4	2	17	3	24	15	18	7	90
8	3	1	14	5	33	10	20	5	91
10	3	1	9	5	39	11	23	5	96
13	2	1	10	4	39	11	17	4	88
15	3	0	12	4	34	16	12	6	87
18	6	1	16	6	36	11	11	5	92
20	5	3	14	2	37	11	13	6	91
23	7	2	15	4	41	9	15	3	96
25	2	0	15	7	31	10	17	3	85
28	9	1	9	5	36	13	16	4	93
30	2	0	12	2	34	13	21	8	92
Total observed	51	12	155	49	413	149	206	61	1,096
Total expected on trihybrid basis	51·36	17·12	154·08	51·36	410·88	136·96	205·50	68·50	1,096
Ratio observed	2·98 :	0·70 :	9·05 :	2·86 :	24·12 :	8·70 :	12·03 :	3·56	
Ratio expected	3 :	1 :	9 :	3 :	24 :	8 :	12 :	4	

$\chi^2=3·72$; $P=0·81$. The fit, therefore, is excellent.



Segregation of size and shape of fruit in F_2.

Grouping all the three grades of purple colour together the following phenotypes and frequencies are obtained :—

	Purple pendent	Purple erect	Non-purple pendent	Non-purple erect	Total
Total observed . . .	618	211	206	61	1,096
Total expected on 9 : 3 : 3 : 1 basis	616·5	205·5	205·5	68·5	1,096
Ratio observed . . .	9·02 :	3·08 :	3·01 :	0·89	
Ratio expected . . .	9 :	3 :	3 :	1	

χ^2=0·972. The fit, therefore, is excellent.

The following segregations were observed in F_3 :—

No. of culture in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
2 cultures from purple gr. III, and pendent-fruited plants	Pure	AABBPP	0	0·66
	Pr. gr. III pendent : Pr. gr. III erect 3 : 1 . . .	AABBPp	2	1·32
2 cultures from purple gr. III, and erect-fruited plants	Pure	AABBpp	2	2
12 cultures from purple gr. II and pendent-fruited plants	Pure	AAbbPP	2	1·33
	Pr. gr. II pendent : Pr. gr. III pendent 3 : 1 . . .	AABbPP	1	2·67
	Pr. gr. II pendent : Pr. gr. II erect 3 : 1 . . .	AAbbPp	1	2·67
	Pr. gr. II pendent : Pr. gr. II erect : Pr. gr. III pendent : Pr. gr. III erect 9 : 3 : 3 : 1 . . .	AABbPp	8	5·34
4 cultures from purple gr. II and erect-fruited plants	Pure	AAbbpp	3	1·3
	Pr. gr. II erect : Pr. gr. III erect 3 : 1 . . .	AAbbPp	1	2·6

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
19 cultures from purple gr. I and pendent-fruited plants	Pr. gr. III pendent : Pr. gr. III pendent : Pr. gr. I pendent 1 : 2 : 1	AaBBPP	1	1·6
	Pr. gr. III pendent : Pr. gr. III erect : Pr. gr. I erect : Non-pr. pendent : Non-pr. erect 3 : 1 : 6	AaBBPp	6	3·2
	Pr. gr. III pendent : Pr. gr. II pendent : Pr. gr. I pendent : Non-pr. pendent 2 : 3 : 1	AaBbPP	0	3·2
	Pr. gr. III pendent : Pr. gr. III erect : Pr. gr. II pendent : Pr. gr. II erect : Pr. gr. I pendent : Pr. gr. I erect : Non-pr. pendent : Non-pr. erect 8 : 4 : 3 : 1	AaBbPp	6	6·4
	Pr. gr. II pendent : Pr. gr. I pendent : Non-pr. pendent 24 : 8 : 12 : 4	AabbPP	1	1·6
	Pr. gr. II pendent : Pr. gr. II erect : Pr. gr. I pendent : Pr. gr. I erect : Non-pr. pendent : Non-pr. erect 1 : 2 : 1	AabbPp	5	3·2
12 cultures from purple gr. I and erect-fruited plants	Pr. gr. III erect : Pr. gr. I erect : Non-pr. erect 1 : 2 : 1	AaBBpp	3	3
	Pr. gr. III erect : Pr. gr. II erect : Pr. gr. I erect : Non-pr. erect 3 : 1 : 4	AaBbpp	7	6
	Pr. gr. II erect : Pr. gr. I erect : Non-pr. erect 1 : 2 : 1	Aabbpp	2	3

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
17 cultures from non-purple pendent-fruited plants	Non-purple pendent . .	aaBBPP aaBbPP aabbPP	5	5·66
	Non-purple pendent : Non-purple erect 3 : 1 . . .	aaBBPp aaBbPp aabbPp	12	11·32
6 cultures from non-purple erect-fruited plants	All non-purple erect . .	aaBBpp aaBbpp aabbpp	6	6

Type 29 × Type 3—F_2.

F_1 plant No.	Purple						Non-purple		Total
	Gr. III pendent	Gr. III erect	Gr. II pendent	Gr. II erect	Gr. I pendent	Gr. I erect	Pendent	Erect	
1	2	1	10	3	32	10	18	5	81
2	1	0	8	5	30	9	18	7	78
3	7	0	12	5	28	12	13	5	82
4	4	0	9	3	38	7	14	3	78
Total observed	14	1	39	16	128	38	63	20	319
Total expected on trihybrid basis	14·7	4·9	44·1	14·7	119·4	39·8	59·7	19·9	317·2
Ratio observed	2·86 :	0·20 :	7·96 :	3·27 :	26·12 :	7·75 :	12·85 :	4·08	
Ratio expected	3 :	1 :	9 :	3 :	24 :	8 :	12 :	4	

$\chi^2=4·7135$; $P=0·69$. The fit, therefore, is good.

Grouping all the grades of purple colour together the following frequencies are obtained :—

	Purple pendent	Purple erect	Non-purple pendent	Non-purple erect	Total
Total observed	181	55	63	20	319
Total expected on 9 : 3 : 3 : 1 basis	179·1	59·7	59·7	19·9	318·4
Ratio observed	9·09 :	2·76 :	3·17 :	1·01	
Ratio expected	9 :	3 :	3 :	1	

$\chi^2=0·57$. The fit, therefore, is excellent.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formula	Frequencies	
			Observed	Expected
2 cultures from purple gr. III and pendent-fruited plants	Pure	AABBPP	1	0·66
	Pr. gr. III pendent : Pr gr. III erect			
	3 : 1 . . .	AABBPp	1	1·32
11 cultures from purple gr. II and pendent-fruited plants	Pure	AAbbPP	2	1·22
	Pr. gr. II pendent : Pr. gr. III pendent			
	3 : 1 . .	AABbPP	3	2·44
	Pr. gr. II pendent : Pr. gr. II erect			
	3 : 1 . . .	AAbbPp	0	2·44
	Pr. gr. II pendent : Pr. gr. II erect			
	9 : 3 :			
	Pr. gr. III pendent : Pr. gr. III erect			
	3 : 1 . . .	AABbPp	6	4·88
4 cultures from purple gr. II and erect-fruited plants	Pure	AAbbpp	2	1·3
	Pr. gr. II erect : Pr. gr. III erect			
	3 : 1 . . .	AABbpp	2	2·6
19 cultures from purple gr. I and pendent-fruited plants	Pr. gr. III pendent : Pr. gr. I pendent			
	1 : 2 :			
	Non-purple pendent			
	1	AaBBPP	2	1·6

Type 29 × Type 3—F_3.

No. of cultures in F_2 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
	Pr. gr. III pendent : Pr. gr. III erect 3 : 1			
	Pr. gr. I pendent : Pr. gr. I erect 6 : 2			
	Non-purple pendent : Non-purple erect 3 : 1 . . .	AaBBPp	2	3·2
	Pr. gr. III pendent : Pr. gr. II pendent 1 : 3			
	Pr. gr. I pendent : Non-purple pendent 8 : 4 . . .	AaBbPP	5	3·2
	Pr. gr. III pendent : Pr. gr. III erect 3 : 1			
	Pr. gr. II pendent : Pr. gr. II erect 9 : 3 :			
	Pr. gr. I pendent : Pr. gr. I erect 24 : 8 :			
	Non-purple pendent : Non-purple erect 12 : 4 . . .	AaBbPp	2	6·4
	Pr. gr. II pendent : Pr. gr. I pendent : Non-purple pendent 1 : 2 : 1 . .	AabbPP	3	1·6
	Pr. gr. II pendent : Pr. gr. II erect : Pr. gr. I pendent 3 : 1 : 6 :			

Type 29 × Type 3—F_3.

No. of cultures in F_2 and nature of parent plants in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
	Pr. gr. I erect : Non-pr. pendent : Non-pr. erect 2 : 3 : 1 . .	AabbPp	5	3·2
4 cultures from purple grade I and erect-fruited plants	Pr. gr. III erect : Pr. gr I erect : Non-purple erect 1 : 2 : 1 . .	AaBBpp	1	1
	Pr. gr. III erect : Pr. gr. II erect 1 : 3			
	Pr. gr. I erect : Non-purple erect 8 : 4 : . . .	AaBbpp	2	2
	Pr. gr. II erect : Pr. gr. I erect : Non-purple erect 1 : 2 : 1 . .	Aabbpp	1	1
16 cultures from non-purple pendent-fruited plants	Non-purple, pendent . . .	aaBBPP aaBbPP aabbPP	6	5·33
	Non-purple pendent : Non-purple erect 3 : 1 . . .	aaBBPp aaBbPp aabbPp	10	10·66
2 cultures from non-purple erect-fruited plants	All non-purple erect . . .	aaBBpp aaBbpp aabbpp	2	2

The F_3 observations confirm the F_2 results on trihybrid basis.

Purple colour and colour of ripe fruit together.
Type 3 × Type 29—F_2.

F_1 plant No.	Purple						Non-purple		Total
	Gr. III red	Gr. III orange	Gr. II red	Gr. II orange	Gr. I red	Gr. I orange	Red	Orange	
3	5	0	11	1	33	15	21	7	93
5	5	1	18	2	30	9	17	7	89
8	3	1	14	5	32	11	18	6	90
10	3	1	13	1	40	9	17	11	95
13	1	2	12	2	43	7	17	4	88
15	3	0	12	4	38	11	16	2	86
18	3	4	15	7	37	10	14	2	92
20	7	1	12	3	33	14	10	8	88
23	8	0	16	3	40	10	10	8	95
25	1	1	20	2	33	7	14	6	84
28	9	1	14	0	31	17	13	7	92
30	2	0	10	3	33	14	18	10	90
Total observed	50	12	167	33	423	134	185	78	1082
Total expected on trihybrid basis	50·7	16·9	152·1	50·7	405·6	135·2	202·8	67·6	1081·6
Deviation	−0·7	−4·9	+14·9	−17·7	+17·4	−1·2	−17·8	+10·4	
Ratio observed	2·96 :	0·71 :	9·89 :	1·95 :	25·03 :	7·93 :	10·95	4·62	
Ratio expected	3 :	1 :	9 :	3 :	24 :	8 :	12 :	4	

$\chi^2=12·99$; $P=0·0723$; and the fit, therefore, is very bad.

The frequencies indicate linkage between purple colour in plant and colour of ripe fruit in it. There is nearly always excess in the frequencies of parental combinations but each character taken singly gives a good 3 : 1 ratio as already seen (pages 230 and 236).

Grouping all purple grades together the following phenotypes and their frequencies are obtained :—

—	Purple plants red fruits	Purple plants orange fruits	Green plants red fruits	Green plants orange fruits	Total
Total observed	640	179	185	78	1082
Total expected on 9 : 3 : 3 : 1 basis .	608·4	202·8	202·8	67·6	1081·6
Deviation	+31·6	−23·8	−17·8	+10·4	
Ratio observed	9·47 :	2·65 :	2·73 :	1·15	
Ratio expected	9 :	3 :	3 :	1	

$\chi^2=7·59$; $P=0·0566$. The fit, therefore, is very bad.

A bad fit, excess in parental combinations, deficiency in single dominants (cross-over classes) and a good 3 : 1 ratio when each character is considered separately indicate linkage. The linkage intensity between factors for purple colour and fruit colour can be estimated by calculating the cross-over value. Applying the 'Product Ratio' method, recommended by Alam [1929], a cross-over value of nearly 44 per cent. is obtained. This value does not suggest a significant linkage.

In F_3 the following segregations were observed :—

No. of cultures in F_2 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
3 cultures from purple gr. III and red-fruited plants	Pure	AABBRR	3	1
	Pr. gr. III red : Pr. gr. III orange 3 : 1 . . .	AABBRr	0	2
1 culture from purple gr. III and orange-fruited plant	Pure	AABBrr	1	1
13 cultures from purple gr. II and red-fruited plants	Pure	AAbbRR	3	1·44
	Pr. gr. II red : Pr. gr. II orange 3 : 1 . . .	AAbbRr	2	2·89
	Pr. gr. II red : Pr. gr. III red 3 : 1 . . .	AABbRR	2	2·89
	Pr. gr. II red : Pr. gr. II orange 9 : 3 Pr. gr. III red : Pr. gr. III orange 3 : 1 . . .	AABbRr	6	5·78
3 cultures from purple gr. II and orange-fruited plants	Pure	AAbbrr	1	1
	Pr. gr. II orange : Pr. gr. III orange 3 : 1 . . .	AABbrr	2	2
23 cultures from purple gr. I and red-fruited plants	Pr. gr. III red : Pr. gr. I red : Non-purple red 1 : 2 : 1 . .	AaBBRR	1	1·9
	Pr. gr. III red : Pr. gr. III orange 3 : 1			

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
	Pr. gr. I red : Fr. gr. I orange 6 : 2 Non-purple red : Non-purple orange 3 : 1	AaBBRr	6	3·8
	Pr. gr. III red : Pr. gr. II red : Pr. gr. I red : Non-pr. red 1 : 3 : 8 : 4	AaBbRR	4	3·8
	Pr. gr. III red : Pr. gr. III orange : Pr. gr. II red : Pr. gr. II orange 3 : 1 : 9 : 3			
	Pr. gr. I red : Pr. gr. I orange : Non-pr. red : Non-pr. orange 24 : 8 : 12 : 4	AaBbRr	6	7·5
	Pr. gr. II red : Pr. gr. I red : Non-pr. red 1 : 2 : 1	AabbRR	1	1·9
	Pr. gr. II red : Pr. gr. II orange : Pr. gr. I red 3 : 1 : 6			
	Pr. gr. I orange : Non-pr. red : Non-pr. orange 2 : 3 : 1	AabbRr	5	3·8
8 cultures from purple gr. I and orange-fruited plants	Pr. gr. III orange : Pr. gr. I orange : Non-pr. orange 1 : 2 : 1	AaBBrr	3	2
	Pr. gr. III orange : Pr. gr. II orange 1 : 3			
	Pr. gr. I orange : Non-pr. orange 8 : 4	AaBbrr	3	4
	Pr. gr. II orange : Pr. gr. I orange : Non-pr. orange 1 : 2 : 1	Aabbrr	2	2
17 cultures from non-purple red-fruited plants	Non-purple, red	aaBBRR aaBbRR aabbRR	2	5·66
	Non-pr. red : Non-pr. orange 3 : 1	aaBBRr aaBbRr aabbRr	15	11·32
6 cultures from non-purple, orange-fruited plants	All non-purple, orange	aaBBrr aaBbrr aabbrr	6	6

Type 29 × Type 3—F_2.

F_1 plant No.	Purple						Non-purple		Total
	Gr. III red	Gr. III orange	Gr. II red	Gr. II orange	Gr. I red	Gr. I orange	Red	Orange	
1	3	0	10	3	37	4	19	4	80
2	1	0	12	1	25	13	16	9	77
3	3	4	12	5	35	6	12	5	82
4	1	3	7	4	30	13	14	3	75
Total observed	8	7	41	13	127	36	61	21	314
Total expected on trihybrid basis	14·7	4·9	44·1	14·7	117·6	39·2	58·8	19·6	313·6
Ratio observed	1·63 :	1·43 :	8·37 :	2·65 :	25·92 :	7·35 :	12·45 :	4·29	
„ expected	3 :	1 :	9 :	3 :	24	8 :	12 :	4	

$\chi^2=5{\cdot}55$; $P=0{\cdot}59$. The fit is good.

Combining all the grades of purple colour together the following frequencies are obtained :—

—	Purple red	Purple orange	Non-purple red	Non-purple orange	Total
Total observed	176	56	61	21	314
„ expected on 9 : 3 : 3 : 1 basis	176·4	58·8	58·8	19·6	313·6
Ratio observed	8·98 :	2·86 :	3·11 :	1·07	
„ expected	9 :	3 :	3 :	1	

$\chi^2=0{\cdot}31$. The fit, therefore, is excellent.

There is no indication of any linkage between colour in plant and colour of ripe fruit as has been observed in cross Type 3× Type 29.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
1 culture from purple gr. III and red-fruited plant	Pure	AABBRR	0	0·33
	Pr. gr. III red : Pr. gr. III orange 3 : 1 . . .	AABBRr	1	0·67
1 culture from purple gr. III and orange-fruited plant	Pure	AABBrr	1	1

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
11 cultures from purple gr. II and red-fruited plants	Pure	AAbbRR	1	1·22
	Pr. gr. II red 3 : Pr. gr. II orange 1	AAbbRr	2	2·44
	Pr. gr. II red 3 : Pr. gr. III red 1	AABbRR	4	2·44
	Pr. gr. II red 9 : Pr. gr. II orange 3 : Pr. gr. III red 3 : Pr. gr. III orange 1	AABbRr	3	4·88
5 cultures from purple gr. II and orange-fruited plants	Pure	AAbbrr	1	1·66
	Pr. gr. II orange 3 : Pr. gr. III orange 1 . . .	AABbrr	4	3·34
20 cultures from purple gr. I and red-fruited plants	Pr. gr. III red 1 : Pr. gr. I red 2 : Non-purple red 1 . .	AaBBRR	1	1·66
	Pr. gr. III red 3 : Pr. gr. III orange 1 : Pr. gr. I red 6 : Pr. gr. I orange 2 : Non-pr. red 3 : Non-pr. orange 1 . .	AaBBRr	2	3·34
	Pr. gr. III red 1 : Pr. gr. II red 3 : Pr. gr. I red 8 : Non-pr. red 4 : Pr. gr. III red 3 : Pr. gr. III orange 1 : Pr. gr. II red 9 : Pr. gr. II orange 3	AaBbRR	3	3·34

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_1	Segregations	Formulæ	Frequencies	
			Observed	Expected
	Pr. gr. I red : Pr. gr. I orange : Non-pr. red : Non-pr. orange 24 : 8 : 12 : 4	AaBbRr	7	6·68
	Pr. gr. II red : Pr. gr. I red : Non-pr. red 1 : 2 : 1	AabbRR	2	1·66
	Pr. gr. II red : Pr. gr. II orange : Pr. gr. I red 3 : 1 : 6			
	Pr. gr. I orange : Non-pr. red : Non-pr. orange 2 : 3 : 1	AabbRr	5	3·34
4 cultures from purple gr. I and orange-fruited plants	Pr. gr. III orange : Pr. gr. I orange : Non-pr. orange 1 : 2 : 1	AaBBrr	2	1
	Pr. gr. III orange : Pr. gr. II orange 1 : 3			
	Pr. gr. I orange : Non-purple orange 8 : 4	AaBbrr	2	2
	Pr. gr. II orange : Pr. gr. I orange : Non-purple orange 1 : 2 : 1	Aabbrr	0	1
14 cultures from non-purple and red-fruited plants	Non-purple, red	aaBBRR aaBbRR aabbRR	4	4·66
	Non-purple red : Non-purple orange 3 : 1	aaBBRr aaBbRr aabbRr	10	9·34
3 cultures from non-purple, orange-fruited plants	All non-purple, orange	aaBBrr aaBbrr aabbrr	3	3

The F_3 observations confirm the F_2 results.

The excess in the number of pure red cultures in the purple grade III and the homozygous purple grade II, and the deficiency of these in the homozygous non-

purple cultures confirm linkage between " colour in plant " and " fruit colour " in Type 3× Type 29.

Fruit-position and colour of ripe fruit together, Type 3 × Type 29—F_2.

F_1 plant No.	Pendent red-fruited	Pendent orange-fruited	Erect red-fruited	Erect orange-fruited	Total
3	52	15	18	8	93
5	51	12	19	7	89
8	47	22	20	1	90
10	57	16	16	6	95
13	56	12	17	3	88
15	45	15	24	2	86
18	50	19	19	4	92
20	48	17	14	9	88
23	63	15	11	6	95
25	48	16	20	0	84
28	52	18	15	7	92
30	46	21	17	6	90
Total observed	615	198	210	59	1082
„ expected on 9 : 3 : 3 : 1 basis	608·4	202·8	202·8	67·6	1081·6
Ratio observed	9·09 :	2·92 :	3·11 :	0·88	
„ expected	9 :	3 :	3 :	1	

$\chi^2=1{\cdot}531$; $P=0{\cdot}6798$. The fit, therefore, is good.

The following segregations were observed in F_3 :—

Number of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
39 cultures from pendent, red-fruited plants	Pure	PPRR	3	4·33
	Pendent red : Pendent orange 3 : 1 . . .	PPRr	4	8·67
	Pendent red : Erect red 3 : 1 . . .	PpRR	8	8·67
	Pendent red : Pendent orange : Erect red : Erect orange 9 : 3 : 3 : 1	PpRr	24	17·32
11 cultures from pendent and orange-fruited plants	Pure	PPrr	3	3·66
	Pendent orange : Erect orange 3 : 1 . . .	Pprr	8	7·32
17 cultures from erect and red-fruited plants	Pure	ppRR	5	5·67
	Erect red : Erect orange 3 : 1 . . .	ppRr	12	11·34
7 cultures from erect, orange-fruited plants	Pure	pprr	7	7

Type 29 × Type 3—F_2.

F_1 plant No.	Pendent red-fruited	Pendent orange-fruited	Erect red-fruited	Erect orange-fruited	Total
1	52	8	17	3	80
2	39	17	15	6	77
3	43	17	19	3	82
4	45	18	7	5	75
Total observed	179	60	58	17	314
,, expected on 9 : 3 : 3 : 1 basis	176·4	58·8	58·8	19·6	313·6
Ratio observed	9·13 :	3·06 :	2·96 :	0·87	
,, expected	9 :	3 :	3 :	1	

$\chi^2 = 0{\cdot}418$. The fit is excellent.

Type 29 × Type 3—F_3.

No. of cultures in F_3 and nature of parent plant in F_2	Segregations	Formulæ	Frequencies	
			Observed	Expected
35 cultures from pendent, red-fruited plants	Pure	PPRR	4	3·87
	Pendent red : Pendent orange 3 : 1 . .	PPRr	12	7·74
	Pendent red : Erect red 3 : 1 . .	PpRR	6	3·87
	Pendent red : Pendent orange : Erect red 9 : 3 :			
	Erect orange 3 : 1 . .	PpRr	13	15·48
10 cultures from pendent and orange-fruited plants	Pure	PPrr	3	3·33
	Pendent orange : Erect orange 3 : 1	Pprr	7	6·67
10 cultures from erect and red-fruited plants	Pure	ppRR	5	3·33
	Erect red : Erect orange 3 : 1 . .	ppRr	5	6·67
3 cultures from erect and orange-fruited plants	Pure	pprr	3	3

The F_3 observations confirm the F_2 results on dihybrid segregation.

Purple colour, fruit-position and colour of ripe fruit together, Type 3 × Type 29—F_2.

F_1 plant No.	PURPLE												NON-PURPLE				Total
	Gr. III pendt. red	Gr. III pendt. orange	Gr. III erect red	Gr. III erect orange	Gr. II pendt. red	Gr. II pendt. orange	Gr. II erect red	Gr. II erect orange	Gr. I pendt. red	Gr. I pendt. orange	Gr. I erect red	Gr. I erect orange	Pendt. red	Pendt. orange	Erect red	Erect orange	
3	5	0	0	0	9	1	2	0	21	8	12	7	17	6	4	1	93
5	4	1	2	0	15	2	3	0	19	5	10	4	14	4	3	3	89
8	3	0	0	1	9	5	5	0	22	11	10	0	13	6	5	0	90
10	2	1	1	0	8	1	5	0	32	6	8	3	15	8	2	3	95
13	1	1	0	1	8	2	4	0	33	6	10	1	14	3	3	1	88
15	3	0	0	0	9	3	3	1	23	10	15	1	10	2	6	0	86
18	3	3	0	1	10	6	5	1	28	8	9	2	9	2	5	0	92
20	3	0	4	0	11	3	1	1	27	9	6	5	7	5	3	3	88
23	7	0	1	0	12	3	4	0	35	6	6	3	10	5	0	3	95
25	1	1	0	0	13	2	7	0	23	7	10	0	11	6	3	0	84
28	8	1	1	0	9	0	5	0	24	12	7	5	11	5	2	2	92
30	2	0	0	0	8	3	2	0	24	10	9	4	12	8	6	2	90
Total observed .	42	8	9	3	121	31	46	3	311	98	112	35	143	60	42	18	1082
Total expected on 4-hybrid basis .	37·8	12·6	12·6	4·2	113·4	37·8	37·8	12·6	304·2	101·4	101·4	33·8	152·1	50·7	50·7	16·9	1080
Deviation . .	+4·2	−4·6	−3·6	−1·2	+7·6	−6·8	+8·2	−9·6	+6·8	−3·4	+10·6	+1·2	−9·1	+9·3	−8·7	+1·1	+2·0
Ratio observed .	10·00	: 1·90 :	2·14 :	0·71 :	28·81 :	7·38 :	10·95 :	0·71 :	74·05 :	23·33 :	26·66 :	8·33 :	34·05 :	14·29 :	10·00 :	4·29	
Ratio expected .	9	: 3 :	3	: 1 :	27	: 9 :	9	: 3 :	72	: 24 :	24	: 8 :	36	: 12 :	12	: 4	

$\chi^2=19{\cdot}55$; $P=0{\cdot}19$. The fit, therefore, is bad.

The expected ratio on 4-hybrid basis should be 81 : 27 : 27 : 27 : 27 : 9 : 9 : 9 : 9 : 9 : 9 : 3 : 3 : 3 : 3 : 1. The expected ratio in the present case, however, looks very different from the above. This is because we have been able to distinguish three genotypes in the purple phenotype and also because of the intensifying factor B, which by itself is without any effect.

It will be seen that the biggest deviations have mostly occurred in those classes where two of the parental characters, either purple colour and red fruit or non-purple colour and orange fruit, which are linked, have appeared together or where they are dissociated.

If the segregation of each of the twelve F_1 plants be looked into, it will be found that there are discrepancies. This is because of the linkage to which we have already referred and also because of the population, about ninety, of each F_1 plant which is very small for considering such a segregation on 4-hybrid basis.

Combining all the purple grades the following frequencies are obtained :—

	Pr. pendt. red	Pr. pendt. orange	Pr. erect red	Pr. erect orange	Non-pr. pendt. red	Non-pr. pendt. orange	Non-pr. erect red	Non-pr. erect orange	Total
Total observed	472	138	168	41	143	60	42	18	1,082
Total expected on tri-hybrid basis . .	456·3	152·1	152·1	50·7	152·1	50·7	50·7	16·9	1,081·6
Deviation .	+15·7	—14·1	+15·9	—9·7	—9·1	+9·3	—8·7	+1·1	..
Ratio observed	27·93 :	8·17 :	9·94 :	2·43 :	8·46 :	3·56 :	2·49 :	1·07	
Ratio expected	27 :	9 :	9 :	3 :	9 :	3 :	3 :	1	

$\chi^2=9{\cdot}180$; $P=0{\cdot}241$. The fit is bad.

It is again the linkage between colour in plant and colour of ripe fruit in it that is responsible for such a bad fit.

Type 29 × Type 3—F_2.

F_1 plant No.	Purple												Non-purple				
	Gr. III pendt. red	Gr. III pendt. orange	Gr. III erect red	Gr. III erect orange	Gr. II pendt. red	Gr. II pendt. orange	Gr. II erect red	Gr. II erect orange	Gr. I pendt. red	Gr. I pendt. orange	Gr. I erect red	Gr. I erect orange	Pendt. red	Pendt. orange	Erect red	Erect orange	Total
1 . . .	2	0	1	0	7	3	3	0	28	3	9	1	15	3	4	1	80
2 . . .	1	0	0	0	6	1	5	0	21	9	5	4	11	7	5	2	77
3 . . .	3	4	0	0	8	4	4	1	23	5	11	1	9	4	4	1	82
4 . . .	1	3	0	0	5	3	2	1	27	10	3	3	12	2	2	1	75
Total observed	7	7	1	0	26	11	14	2	99	27	28	9	47	16	15	5	314
Total expected on 4-hybrid basis	11·03	3·67	3·67	1·22	33·09	11·03	11·03	3·67	88·20	29·40	29·40	9·80	44·10	14·70	14·70	4·90	313·6
Ratio observed	5·74	: 5·74	: 0·82	: 0	: 21·31	: 9·02	: 11·48	: 1·64	: 81·15	: 22·13	: 22·95	: 7·38	: 38·52	: 13·11	: 12·29	: 4·10	
Ratio expected	9	: 3	: 3	: 1	: 27	: 9	: 9	: 3	: 72	: 24	: 24	: 8	: 36	: 12	: 12	: 4	

$\chi^2 = 12{\cdot}67$; $P = 0{\cdot}627$. The fit is good.

Combining all the purple grades the following frequencies are obtained :—

	Pr. pendt. red	Pr. pendt. orange	Pr. erect red	Pr. erect orange	Non-pr. pendt. red	Non-pr. pendt. orange	Non-pr. erect red	Non-pr. erect orange	Total
Total observed	132	45	43	11	47	16	15	5	314
Total expected on tri-hybrid basis	132·30	44·10	44·10	14·70	44·10	14·70	14·70	4·90	313·6
Ratio observed	26·94 :	9·18 :	8·78 :	2·24 :	9·59 :	3·26 :	3·06 :	1·02	
Ratio expected	27 :	9 :	9 :	3 :	9 :	3 :	3 :	1	

$\chi^2=1·29$; $P=0·98$. The fit, therefore, is excellent.

The F_3 observations have not been presented here as there were rather big discrepancies between the observed and expected results. This was principally due to the number of cultures, 74 and 58 in Type 3 × Type 29 and the reciprocal respectively which was too small to consider segregations on 4-hybrid basis.

Fruit-length.

The two parents were very distinct as regards their fruit-length, the mean fruit-length of Type 3 being 2·5 cm. with a probable error of 0 in 1928 and 2·5 cm. with a probable error of ±0·067 in 1929, and that of Type 29 parent 11·02±0·133 cm. in 1928 and 10·91±0·067 cm. in 1929. The F_1, which was grown in the year 1928, had a mean fruit-length of 5·32±0·06 cm. In other words, F_1 was nearly intermediate for this character. The standard deviation for Type 3 is unusually low while that for Type 29 is rather high.

TABLE V.

Type 3 × Type 29.

Frequency distribution of length of fruits.

No.	Parental value	Class centres in centimetres												Total number of fruits	Mean	Standard deviation	Co-efficient of variation
		1·5	2·5	3·5	4·5	5·5	6·5	7·5	8·5	9·5	10·5	11·5	12·5				
Type 3 parent, 1928-29	...	...	25	..	..	...	...	...	...	...	...	...	...	25	2·50±0	0	0
Type 3 parent, 1929-30	...	1	45	1	...	...	...	...	...	...	...	...	...	47	2·50±·021	·21±·014	8·40± ·583
Type 29 parent, 1928-29	...	...	...	...	...	...	...	...	1	4	9	9	6	29	11·02±·133	1·06±·09	9·62± ·86
Type 29 parent, 1929-30	...	...	...	...	...	...	...	...	1	9	29	30	6	75	10·91±·067	·86±·047	7·88± ·436
T. 3 × T. 29 F_1, 1928-29	...	...	...	...	6	21	1	...	...	...	...	...	...	28	5·32±·06	·47±·04	8·83± ·80
T. 3 × T. 29 F_2, 1929-30	...	1	32	120	206	224	200	104	72	25	11	2	...	997	5·75±·037	1·74±·026	30·26± ·49
15—80 × T. 29 F_3, 1930-31	2·18	...	9	18	...	...	...	...	...	...	...	...	...	27	2·85±·06	·47±·04	16·49±1·515
18—36 ,, F_3, 1930-31	2·40	...	28	2	...	...	...	...	...	...	...	...	...	30	2·57±·03	·25±·02	9·73± ·85
8—35 ,, F_3, 1930-31	2·46	...	36	3	5	...	...	...	...	...	...	...	...	44	2·79±·07	·65±·05	23·29±1·76
18—93 ,, F_3, 1930-31	2·5	...	6	13	6	...	...	...	...	...	...	...	...	25	3·55±·09	·69±·07	19·44±1·91
13—42 ,, F_3, 1930-31	2·64	...	23	2	...	...	...	...	...	...	...	...	...	25	2·63±·04	·27±·03	10·26± ·98
10—79 ,, F_3, 1930-31	2·88	...	1	13	13	5	3	2	0	1	...	...	...	38	4·68±·13	1·17±·09	25·00±2·04
25—76 ,, F_3, 1930-31	3·10	...	22	23	7	...	...	...	...	...	...	...	...	62	3·26±·06	·65±·04	19·94±1·26
8—84 ,, F_3, 1930-31	3·12	...	22	30	6	...	...	...	...	...	...	...	...	58	3·22±·06	·64±·04	19·88±1·30
18—28 ,, F_3, 1930-31	4·43	...	...	18	8	4	...	...	...	...	...	...	...	30	4·08±·09	·72±·06	17·67±1·58
8—86 ,, F_3, 1930-31	4·64	...	4	13	9	11	3	0	2	0	1	...	...	43	4·73±·17	1·66±·12	35·09±2·85
13—1 ,, F_3, 1930-31	6·34	...	...	...	...	9	24	2	...	...	...	...	...	35	6·35±·06	·52±·04	8·24± ·67
8—36 ,, F_3, 1930-31	6·72	...	...	1	2	3	3	3	2	2	1	1	...	18	7·17±·34	2·13±·24	29·71±3·60
8—83 ,, F_3, 1930-31	7·78	...	...	...	4	6	10	3	2	...	...	...	...	25	6·22±·15	1·11±·11	17·84±1·74
20—10 ,, F_3, 1930-31	9·76	...	...	...	...	...	2	5	5	3	2	...	...	17	8·43±·20	1·21±·14	14·35±1·69
3—7 ,, F_3, 1930-31	10·10	...	...	...	...	1	0	4	8	5	2	3	1	24	9·13±·21	1·56±·15	17·09±1·39
10—37 ,, F_3, 1930-31	11·34	...	...	2	1	2	11	6	4	3	5	2	...	36	7·75±·23	2·04±·16	26·32±2·72

In F_2 the mean fruit-length was $5{\cdot}75 \pm 0{\cdot}037$ cm. and was very close to the F_1 mean. The range of variability was great (Plate XXI) starting from the lowest range of the small-fruited parent, and failing by only one class to reach the highest range of the long-fruited parent (Fig. 1 and Table V). The standard deviation was very high and so was the coefficient of variability. The parental forms were realized in a fairly large proportion (Table V). The frequency curve for F_2, which is normal and unimodal, is shown along with the curves for the parents and the F_1 (Fig. 1).

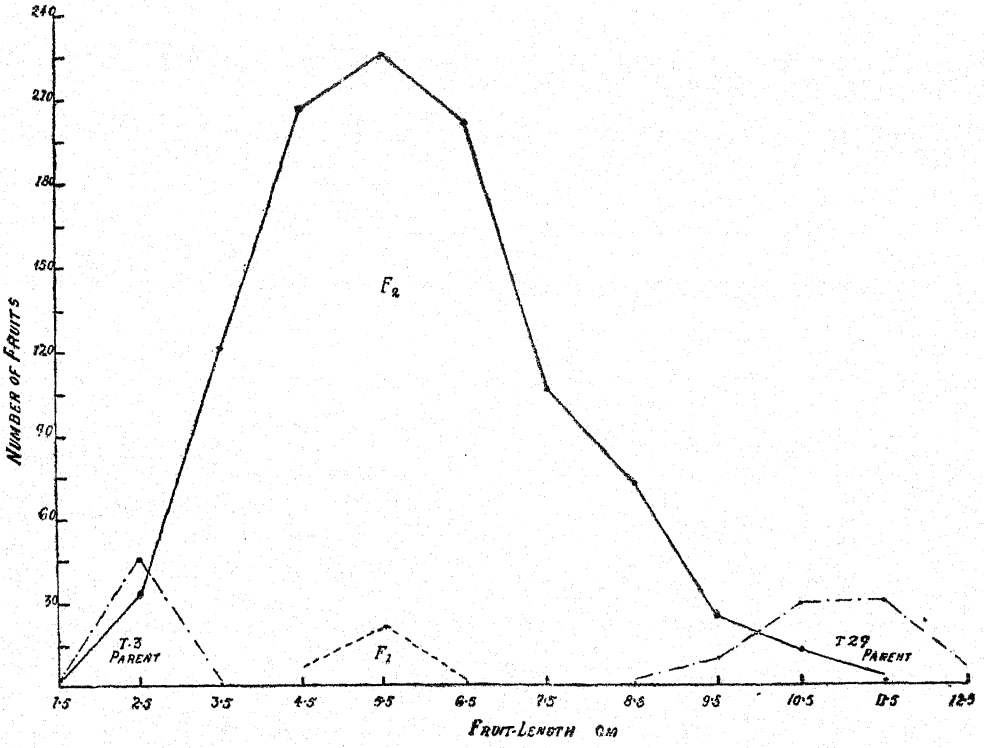


Fig. 1.—Frequency curves for fruit-length.

In F_3, the mean length of the short-fruited cultures was, is most cases, close to the parental value with a very low standard deviation. Cultures with fruits intermediate in length (like F_1 or very nearly so) gave nearly the same mean length but showed quite high variability like the F_2. Cultures from long-fruited plants, however, gave in all cases a lower mean value with a high standard deviation (Table V).

As to the difference in the number of factors between the parents for this character nothing can definitely be said but the realization of the parental forms

in F_2 in a large proportion suggests that the parents do not differ by many factors. The F_2 frequencies when adjusted and added up, as shown below, suggest a close agreement to segregation on tri-hybrid basis.

	Range of Type 3			Range of F_1				Range of Type 29				
	1	32	120	206	224	200	104	72	25	11	2	=997
			60 60									
Observed frequencies of parental and F_1 range		93			794				110			=997
Expected frequencies on 1 : 6 : 15 : 20 : 15 : 6 : 1 tri-hybrid basis	15·55	93·30		233·25	311	233·25		93·30	15·55			=995·2
Expected frequencies of parental and F_1 range		108·85			777·50				108·85			
Deviation . . .		15·85			16·50				1·15			

It will be observed that the entire F_2 population has been grouped into three classes representing the two parents and the F_1. The justification for splitting 120, the frequency in the 3·5 cm. class (Table V), lies in the fact that the F_1 population is small and that had it been bigger we might almost certainly have noticed some F_1 individuals in that class so that the frequency in this class would be a mixture of individuals of both the groups. It will also be seen that the frequency, 104, in the 7·5 cm. class is entirely included in the middle class. Type 29, even with its large population, has failed to appear in this class, while F_1 with a small population is as much near to it as Type 29 and had the F_1 population been bigger some F_1 individuals might be expected in that class.

Castle's formula [1926] also indicates a 3-factor difference between the parents. The actual value of "n" (number of factors concerned) in this case as derived by the said formula is 3·1.

On the other hand there is evidence of linkage between purple colour and short fruit and non-purple colour and long fruit as will be seen from Fig. 2 and Table VI. The table shows a 3 purple : 1 non-purple ratio in the classes 5·01-6·00 and 6·01-7·00 cm., and that the proportion of fruits with purplish tinge increases towards the lower extremity till at the two lowermost classes no non-purple individual is seen. Similarly the proportion of fruits without purple tinge increases towards the higher extremity till the last class is reached where no purple individual is observed, while on theory the ratio of purples to non-purples in each class should be 3 : 1. This indicates that the shorter fruits are more often associated

with the purple colour character than with the non-purple and *vice versâ*. In other words, the two characters are linked.

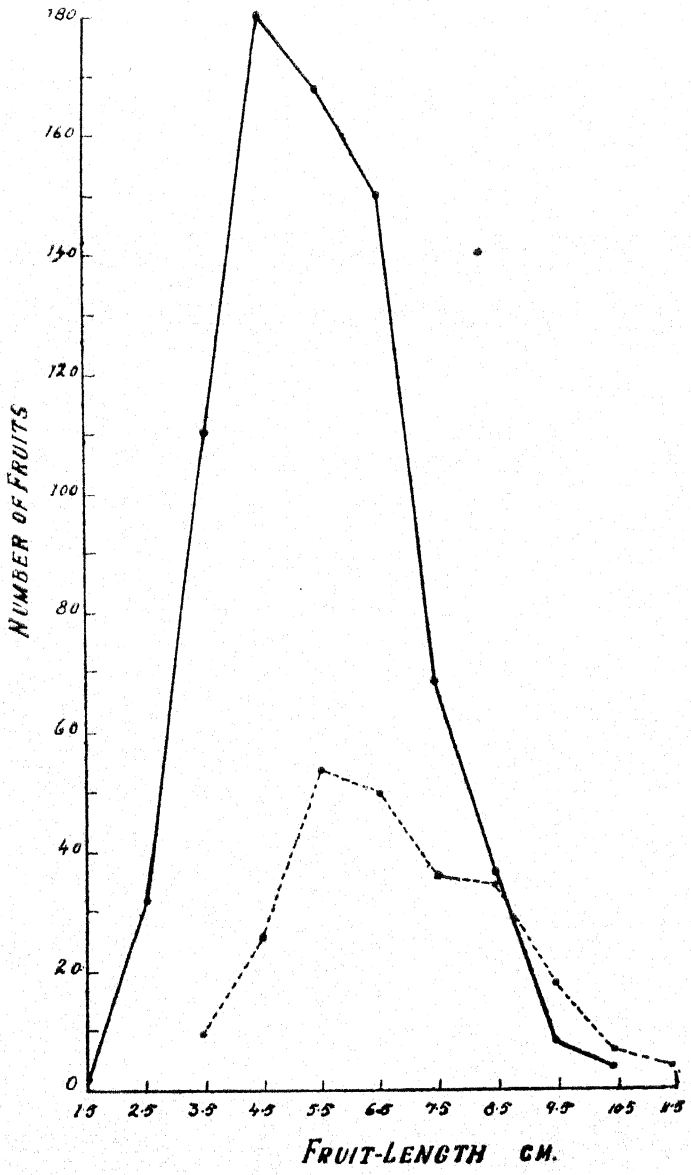


Fig. 2.—Frequency curves for length of fruits of purple and non-purple plants in F_2.
Continuous line : fruits of purple plants.
Broken line : fruits of non-purple plants.

Looking to the original F_2 curve for the combined frequencies (Fig. 1) it will be seen that from the class 7·5 cm. the curve suddenly departs to the right which is an indication that from this class longer fruits are appearing. If, therefore, we dissect the F_2 frequencies at this point, the lower portion representing smaller fruits (Type 3 and F_1) and the upper the longer (Type 29) and find the ratio of the former to the latter we get a fairly close approximation to a 3 : 1 ratio as shown below :—

	Range of small and intermediate fruits (like Type 3 and F_1)	Range of long fruits (like Type 29)	
	1 32 120 206 224 200	104 72 25 11 2	=997
Total observed . . .	783	214	=997
Total expected on 3 : 1 basis .	747·75	249·25	=997
Deviation / Probable Error . . .	$=\frac{35\cdot25}{9\cdot22}$		=3·82

The fit, of course, is rather bad but good enough to indicate a segregation on 3 : 1 basis in a quantitative character as the present one.

This interpretation that the segregation in this character is on a 3 : 1 basis is in agreement with the one advanced previously wherein we have an indication of a difference of three factors between the parents for this character. The only difference between the two interpretations lies in the fact that in interpreting a segregation on a 3 : 1 ratio we have grouped small and intermediate fruits together against the long ones while in the other case we have grouped the whole population in three classes representing the Type 3, the F_1, and the Type 29 fruits. The 3 : 1 ratio may really be a trihybrid 48 : 16 ratio.

The fruit-length seems further linked with the fruit-position. The longer fruits are much more frequently associated with the pendent position than with the erect and the shorter with the erect than with the pendent. Table VII, in which the F_2 frequencies of pendent and erect fruits together with their ratio in each class of fruit-length have been entered, shows that towards the lower extremity of the range the proportion of pendent fruits is low while towards the upper it is high and that in the two uppermost classes no erect fruit is found. The curve and the modal class for the pendent fruits will be seen to have shifted up (Fig. 3) indicating greater fruit-length. On the basis of independent segregation the proportion of pendent to erect fruits in each class should be as 3 : 1 and the curves for the pendent and erect fruits should be in close agreement.

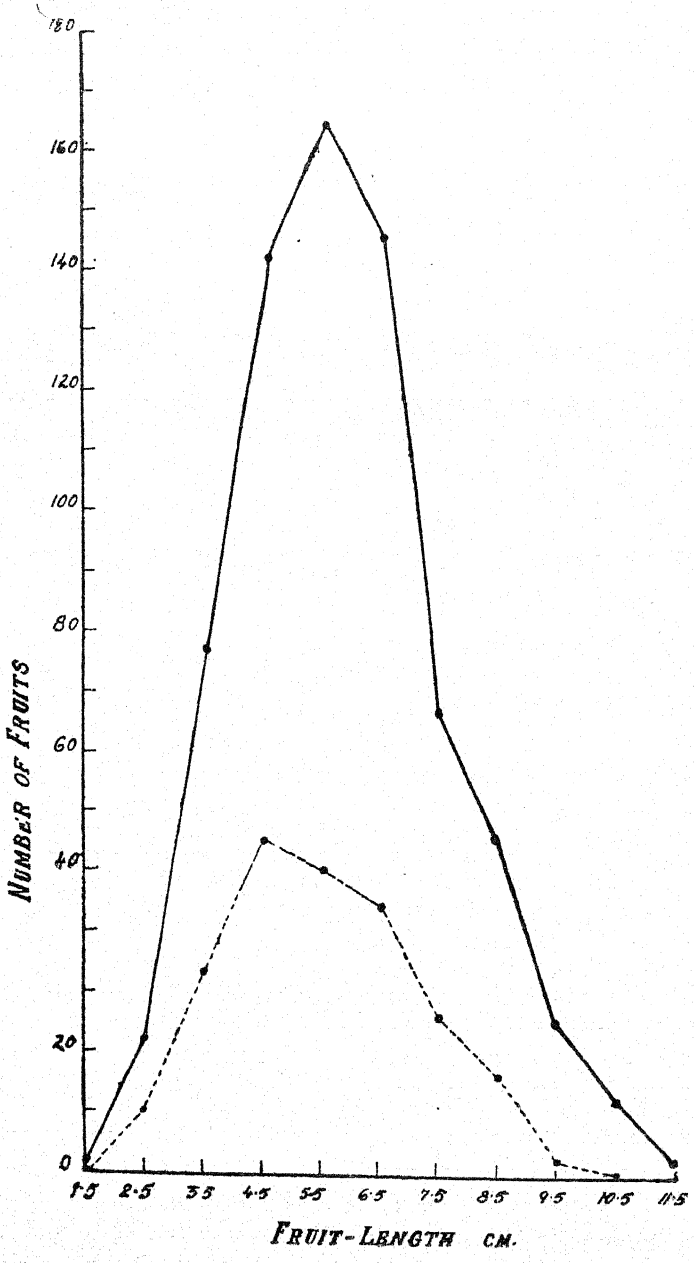


Fig. 3.

Frequency curves for length of pendent and erect fruits in F_2.
Continuous line: pendent fruits.
Broken line: erect fruits.

TABLE VI.

Frequencies of fruits of purple and non-purple plants in F_2 and their ratio in each class for fruit-length.

Class centres in cm.	1·5	2·5	3·5	4·5	5·5	6·5	7·5	8·5	9·5	10·5	11·5	Total
	Pr.*N-pr.†	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.
Frequencies of fruits from purple and non-purple plants	1 0	32 0	110 9	180 26	168 54	150 50	69 36	36 35	8 18	4 7	0 2	758 237
Ratio of fruits from purple to fruits from non-purple plants	1 : 0	32 : 0	12·2 : 1	6·8 : 1	3·1 : 1	3·0 : 1	1·9 : 1	1·03 : 1	1 : 2·2	1 : 1·7	0 : 2	3·19 : 1

* Pr. = Purple.
† N-pr. = Non-purple.

TABLE VII.

Frequencies of pendent and erect fruits in F_2 and their ratio in each class of fruit-length.

Class centres in cm.	1·5	2·5	3·5	4·5	5·5	6·5	7·5	8·5	9·5	10·5	11·5	Total
	P.* E.†	P. E.	P. E.	P. E.	P. E.	P, E.	P. E.	P. E.	P. E.	P. E.	P. E.	P. E.
Frequencies of pendent and erect fruits	1 0	22 10	87 33	152 55	175 50	156 44	77 26	56 16	25 2	12 0	2 0	765 236
Ratio of pendent to erect fruit	1 : 0	2·20 : 1	2·64 : 1	2·76 : 1	3·50 : 1	3·55 : 1	2·96 : 1	3·50 : 1	12·50 : 1	12 : 0	2 : 0	3·2 : 1

* P. = Pendent.
† E. = Erect.

Fruit-thickness.

The fruit of Type 3 is thick and has a mean thickness of 1·60 ± 0·007 cm. while that of Type 29 is thin with a mean thickness of 1·22 ± 0·006 cm. The F_1 fruit was thicker than that of the thick-fruited parent with a mean thickne s of 1·77 ± 0·003 cm. thereby showing dominance of thick fruit over the thin and manifesting heterosis (Table VIII and Plate XV1). The parents have a low and nearly the same standard deviation while F_1 has even less.

The mean fruit-th ickness of F_2 was 1·70 ± 0·005 cm., that is, a little less than F_1 but yet greater than that of the thick-fruited parent.

The F_2 showed increased variability over F_1, the standard deviation and coefficient of variation being $0{\cdot}225 \pm 0{\cdot}003$ and $13{\cdot}25 \pm 0{\cdot}202$ respectively, both being high. The range of variation was also large extending beyond the extreme limits of either parent (Table VIII and Fig. 4).

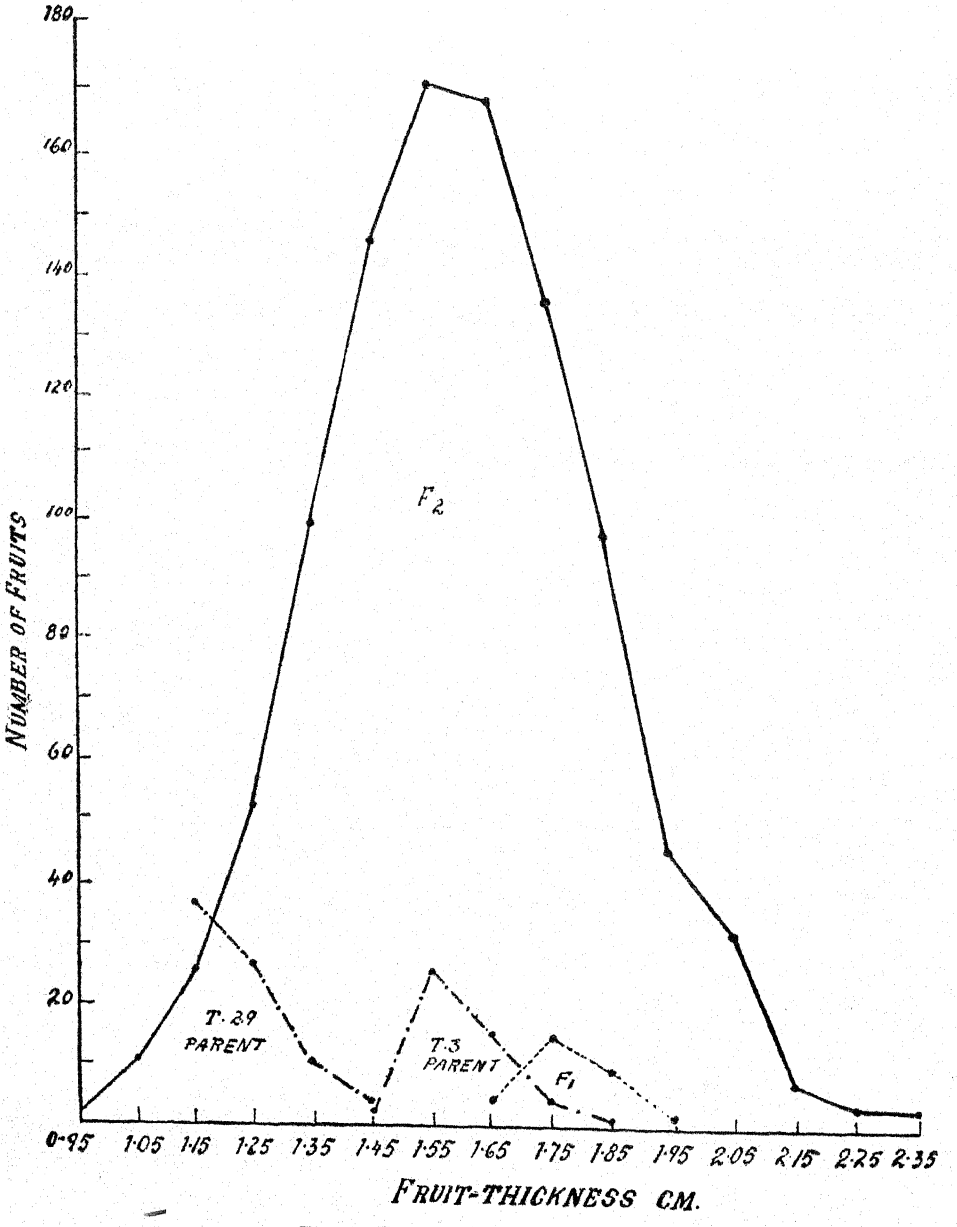


Fig. 4—Frequency curves for fruit-thickness.

TABLE VIII.

Type 3 × Type 29

Frequency distribution of thickness of fruits.

No.	Parental value	Class centres in centimetres																Total number of fruits	Mean	Standard deviation	Co-efficient of variation
		0·95	1·05	1·15	1·25	1·35	1·45	1·55	1·65	1·75	1·85	1·95	2·05	2·15	2·25	2·35	2·45				
Type 3 parent, 1928-29 .	..	..	..	..	..	1	2	12	8	1	..	..	..	..	..	..	..	24	1·58 ± ·01	·08 ± ·008	5·22 ± ·503
„ „ „ 1929-30 .	..	..	..	..	..	..	2	25	15	4	1	..	..	..	..	..	..	47	1·60 ± ·007	·08 ± ·005	4·94 ± ·34
Type 29 parent, 1928-29 .	..	..	2	13	10	3	..	..	..	..	..	..	..	..	..	..	..	28	1·20 ± ·01	·08 ± ·007	6·42 ± ·58
„ „ „ 1929-30 .	..	..	..	36	26	10	3	..	..	..	..	..	..	..	..	..	..	75	1·22 ± ·006	·08 ± ·004	6·88 ± ·38
T. 3 × T. 29 F_1, 1928-29.	..	..	..	..	..	..	..	..	4	14	9	1	..	..	..	..	..	28	1·77 ± ·003	·03 ± ·002	1·47 ± ·13
„ „ F_2, 1929-30 .	..	1	10	25	53	99	145	171	168	135	97	45	32	7	3	3	..	994	1·70 ± ·005	·22 ± ·003	13·25 ± ·202
„ „ F_3, 1930-31.	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
13—1 . . .	·92	5	21	8	1	..	..	..	..	..	..	..	..	..	..	..	..	35	1·07 ± ·003	·02 ± ·002	2·03 ± ·16
10—37 . .	1·30	..	1	4	8	10	8	4	2	1	..	..	..	..	..	..	..	38	1·37 ± ·024	·22 ± ·017	16·06 ± 1·27
13—83 . .	1·34	1	5	6	4	10	5	8	6	3	2	..	..	..	..	..	..	50	1·40 ± ·02	·22 ± ·01	15·71 ± 1·07
20—10 . .	1·36	..	..	1	0	3	8	3	1	1	..	..	..	..	..	..	..	17	1·46 ± ·03	·16 ± ·02	10·96 ± 1·29
13—42 . .	1·48	..	..	..	..	..	..	..	2	11	7	5	..	..	..	..	..	25	1·81 ± ·009	·07 ± ·007	3·64 ± ·35
3—7 . .	1·50	..	..	..	..	4	5	6	5	2	0	2	..	..	..	..	..	24	1·57 ± ·07	·53 ± ·05	33·76 ± 2·63
8—83 . .	1·54	..	..	..	..	..	..	2	3	3	6	5	4	2	..	..	..	25	1·87 ± ·022	·16 ± ·015	8·56 ± ·82
18—93 . .	1·60	..	..	..	..	..	..	..	..	..	5	1	2	7	4	3	3	25	2·15 ± ·03	·19 ± ·02	8·98 ± ·86
8—35 . .	1·66	..	..	..	..	..	..	2	3	14	15	8	4	3	1	1	..	51	1·87 ± ·014	·15 ± ·01	8·18 ± ·55
25—76 . .	1·72	..	..	..	..	..	..	9	16	16	12	6	1	1	..	..	..	61	1·74 ± ·012	·14 ± ·009	8·27 ± ·51
8—84 . .	1·82	..	..	..	..	..	..	1	2	5	11	11	15	7	7	2	1	62	2·00 ± ·02	·19 ± ·01	9·40 ± ·58
8—36 . .	1·86	..	..	..	..	1	1	1	2	4	1	2	3	2	1	..	..	18	1·84 ± ·04	·24 ± ·03	13·32 ± 1·52
18—28 . .	2·06	..	..	..	..	..	..	..	1	1	3	8	7	7	3	..	..	30	2·03 ± ·02	·14 ± ·01	7·09 ± ·62
8—86 . .	2·12	..	1	0	1	1	2	4	3	5	7	10	5	3	0	1	..	43	1·82 ± ·03	·26 ± ·02	14·13 ± 1·04

In F_3 the thin-fruited cultures had a mean thickness more or less like their parents. The standard deviation in one case was very low while in others it was nearly as high as in F_2.

Out of all the thick-fruited cultures some gave nearly the same mean thickness as their parents while others gave much higher and only one culture gave a considerably lower mean thickness.

Judging from the population of F_2 individuals, appearing in the range of thin-fruited parent, it does not seem likely that the number of factors by which the parents differ for this character is great. On the contrary, there seems, in all probability, only a single factor difference. That this is so will be seen from the fact that the ratio of thin to thick fruits is nearly as 1 : 3 as shown below :—

	Range of Type 29	Combined range of Type 3 and F_1	
	1 10 25 53 99	145 171 168 135 97 45 32 7 3 3	= 994
	72·5	72·5	
Observed frequencies in the range of thin-fruited parent and combined range of thick-fruited parent and F_1	260·5	733·5	= 994
Expected frequencies on 1 : 3 basis	248·5	745·5	= 994
Ratio observed	0·98 :	3·02	
Ratio expected	1 :	3	

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{12}{9{\cdot}21} = 1{\cdot}30$$

The fit, therefore, is good.

The frequency, 145, in class 1·45 cm., (Table VIII) is included half and half into each of the two ranges as this class is represented by both the types of fruits.

Another fact which corroborates the inheritance of fruit-thickness on a monohybrid basis is the linkage between "thin fruit" and the character of calyx "enclosing fruit-base" and between thick fruit and calyx "not enclosing fruit-base".

as shown below. For the following classification fruits measuring 1·45 cm. and below were considered as thin and those measuring 1·46 cm. upwards as thick.

	Thick fruit, calyx "not enclosing fruit-base"	Thick fruit, calyx "enclosing fruit-base"	Thin fruit, calyx "not enclosing fruit-base"	Thin fruit, calyx "enclosing fruit-base"	
Total observed	708	63	79	148	=998
Total expected on 9 : 3 : 3 : 1 basis	561·33	187·11	187·11	62·37	=997·92
Deviation	+146·67	—124·11	—108·11	+85·63	

The excess in the parental combinations is very high and suggests a significant linkage between thick fruit and unenclosed fruit-base and thin fruit and enclosed fruit-base. Besides, each character taken singly gives a fairly good 3 : 1 ratio as already shown (pages 272 and 242). The actual cross-over value, as calculated by the 'Product Ratio' method, comes to 16 per cent. which indicates a very high linkage. The above facts, therefore, are quite in favour of a monohybrid segregation on a 3 : 1 basis in this character.

Petal-length.

The two parents differ sharply for this character. The mean petal-length in Type 3 in 1928 and 1929 was respectively 9·77±0·091 mm. and 8·84±0·03 mm. and that in Type 29 for the same two years was respectively 16·15±0·089 mm. and 14·48 ±0·07 mm. The differences in the means for the two years are big enough to be statistically significant in both the types. The difference in the former case being about 9·8 times its probable error while in the latter it is about 15·1 times its probable error. This variability in the means obtained in these two years is, in our opinion, due both to the small parental populations in 1928 and seasonal and soil differences in the two years. In F_1 the mean petal-length was 11·03±0·118 mm. and F_1 was, therefore, nearly intermediate between the parents in this respect. In F_2 the mean petal-length was 12·13±0·022 mm. that is, the F_2 also showed, like the F_1, an intermediacy for petal-length and the range reached the lowest and the highest extremities of the smaller and the larger parents respectively. The parental forms were recovered in quite a large proportion (Table IX).

In F_3, cultures with low parental value bred true showing a small amount of variability. Those with value like the F_1 or F_2 gave nearly the same mean length but showed rather a high variability. Cultures with a high parental mean value gave rather a lower mean value and a high variability.

The F_2 frequency curve (Fig. 5) is unimodal and nearly symmetrical. It does not throw any light on the number of factors involved for this character. How-

ever, the realization of parental forms in F_2 in a large proportion suggests that the parents do not differ by many factors for this character.

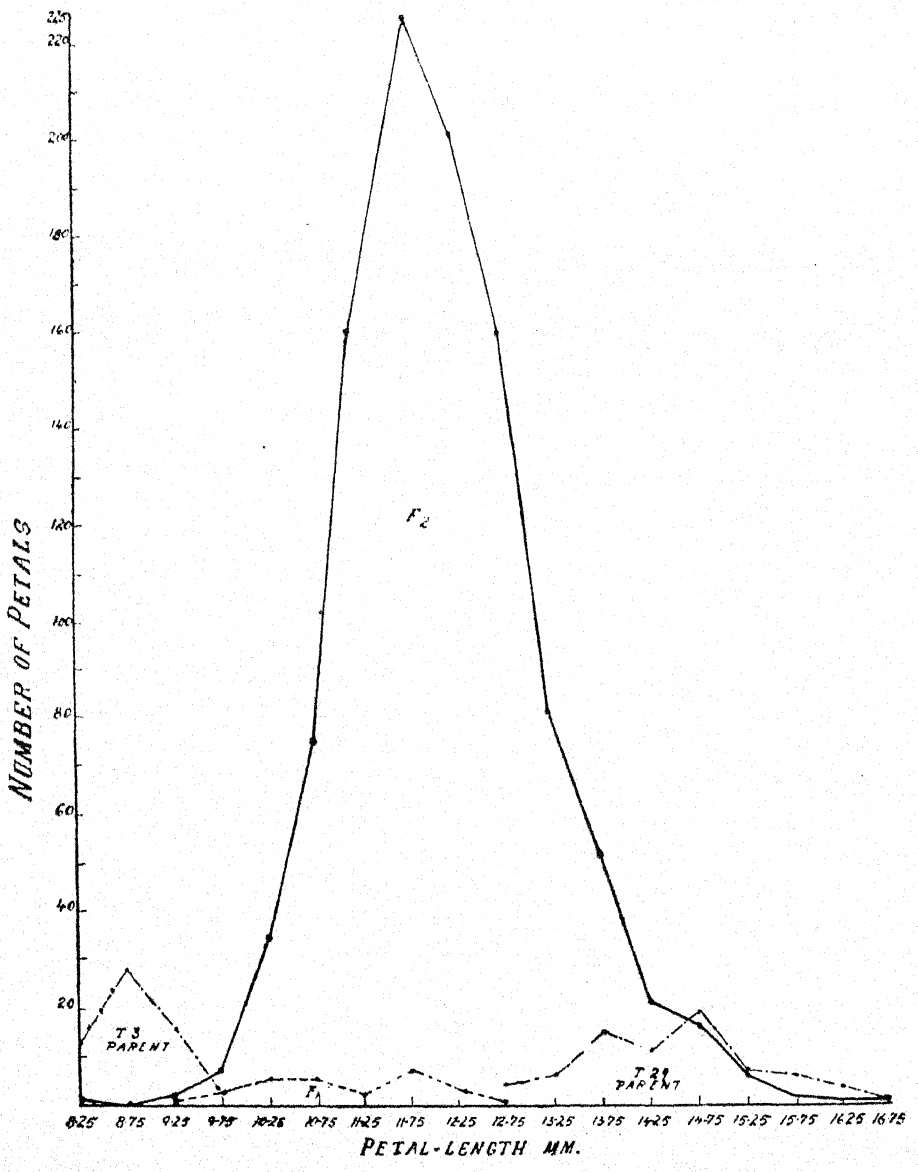


Fig. 5.—Frequency curves for petal-length.

TABLE IX.

Type 3×Type 29.

Frequency distribution of length of petals.

No.	Parental value	CLASS CENTRES IN MILLIMETRES																		Total No. of petals	Mean	Standard deviation	Co-efficient of variation
		8·25	8·75	9·25	9·75	10·25	10·75	11·25	11·75	12·25	12·75	13·25	13·75	14·25	14·75	15·25	15·75	16·25	16·75				
Type 3 parent 1928-29	..	1	3	12	11	1	1	..	..	..	..	..	..	..	..	..	..	..	..	29	9·77±·091	·73±·06	7·47±·66
Type 3 parent 1929-30	..	13	28	16	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	61	8·84±·03	·42±·02	4·75±·29
Type 29 parent 1928-29	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	5	13	9	..	28	16·15±·089	·70±·06	4·34±·39
Type 29 parent 1929-30	..	..	..	..	..	..	..	..	..	..	4	6	15	11	19	7	6	4	1	73	14·48±·07	·93±·05	6·42±·36
T. 3×T. 29 F_1 1928-29	..	..	..	1	3	5	5	2	7	3	1	..	..	..	..	..	..	..	..	27	11·03±·118	·91±·08	8·25±·76
T. 3×T. 29 F_2 1929-30	..	1	0	2	7	34	74	160	225	200	160	81	52	22	17	6	2	1	1	1045	12·13±·022	1·06±·015	8·74±·13
T. 3×T. 29 F_3 1930-31	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
23—56 .	9·84	1	1	3	8	4	4	..	..	..	..	..	..	..	..	..	..	..	..	21	9·84±·10	·648±·07	6·58±·69
10—71 .	10·74	3	6	7	6	1	..	..	..	..	..	..	..	..	..	..	..	..	..	22	9·18±·08	·54±·06	5·98±·61
5—18 .	10·84	..	..	..	6	6	5	6	4	1	1	..	..	..	..	..	..	..	..	29	10·80±·10	·81±·07	7·55±·67
13—71 .	11·83	1	0	2	4	7	13	14	12	2	3	..	..	..	..	..	..	..	..	58	10·94±·08	·95±·06	8·68±·55
8—86 .	12·00	..	..	..	..	8	11	12	9	6	2	2	3	..	..	..	..	..	..	53	11·47±·09	·94±·06	8·20±·54
13—42 .	12·46	..	..	4	14	12	13	6	5	1	..	..	..	..	..	..	..	..	..	55	10·45±·07	·74±·05	7·04±·45
8—83 .	12·52	..	..	..	2	4	3	6	3	9	2	..	..	..	..	..	..	..	..	29	11·42±·11	·88±·08	7·71±·69
5—8 .	12·52	..	..	..	..	1	3	3	6	2	2	..	..	..	..	..	..	..	..	17	11·57±·11	·68±·08	5·92±·68
3—3 .	15·22	..	..	..	..	1	0	1	4	6	2	3	5	1	1	..	..	..	..	29	12·80±·12	·97±·09	7·61±·68
3—24 .	15·60	..	..	..	..	..	..	..	1	1	3	5	3	4	3	..	..	..	..	20	13·55±·12	·83±·09	6·01±·64

TABLE X.

Frequencies of purple and non-purple petals in F_2 and their ratio in each class for petal-length.

Class centres in mm.	8·75	9·25	9·75	10·25	10·75	11·25	11·75	12·25	12·75
	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.
Frequencies of purple and non-purple petals	1 0	3 0	7 0	31 3	71 6	150 13	195 29	155 45	107 56
Ratio of purple to non-purple petals	1 : 0	3 : 0	7 : 0	10·33 : 1	11·83 : 1	11·54 : 1	6·72 : 1	3·44 : 1	1·91 : 1

TABLE X—*contd.*

Frequencies of purple and non-purple petals in F_2 and their ratio in each class for petal-length.

Class centre in mm.	13·25	13·75	14·25	14·75	15·25	15·75	16·25	16·75	Total
	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr.	Pr. N-pr	Pr. N-pr.
Frequencies of purple and non-purple petals	46 37	25 26	6 16	6 11	0 6	0 2	0 0	1 1	804 251
Ratio of purple to non-purple petals	1·24 : 1	1 : 1·04	1 : 2·66	1 : 1·83	0 : 6	0 : 2	0 : 0	1 : 1	3·20 : 1

That there is linkage between petal-length and colour in plant is evident from Table X wherein the F_2 frequencies of purple and non-purple petals together with their proportions as occurring in each of the classes for petal-length are given. It will be seen from the table that in the class 12·25 mm. the proportion of purple to non-purple petals is 3·44 : 1 and that towards the lower extremity it generally goes on increasing until the last three lowest classes are reached wherein no non-purple individual is present. In the same way the proportion of non-purple individuals generally increases as we go up the range from the 12·25 mm. class till we reach the end classes in which barring a solitary purple individual in the last class no purple petals are observed. Theoretically we expect in each class purple and non-purple petals in the ratio of 3 : 1.

Fig. 6, in which the frequency curves for purple and non-purple petals are plotted, shows that the curve as well as the mode for the latter has shifted up which is an indication of greater petal-length.

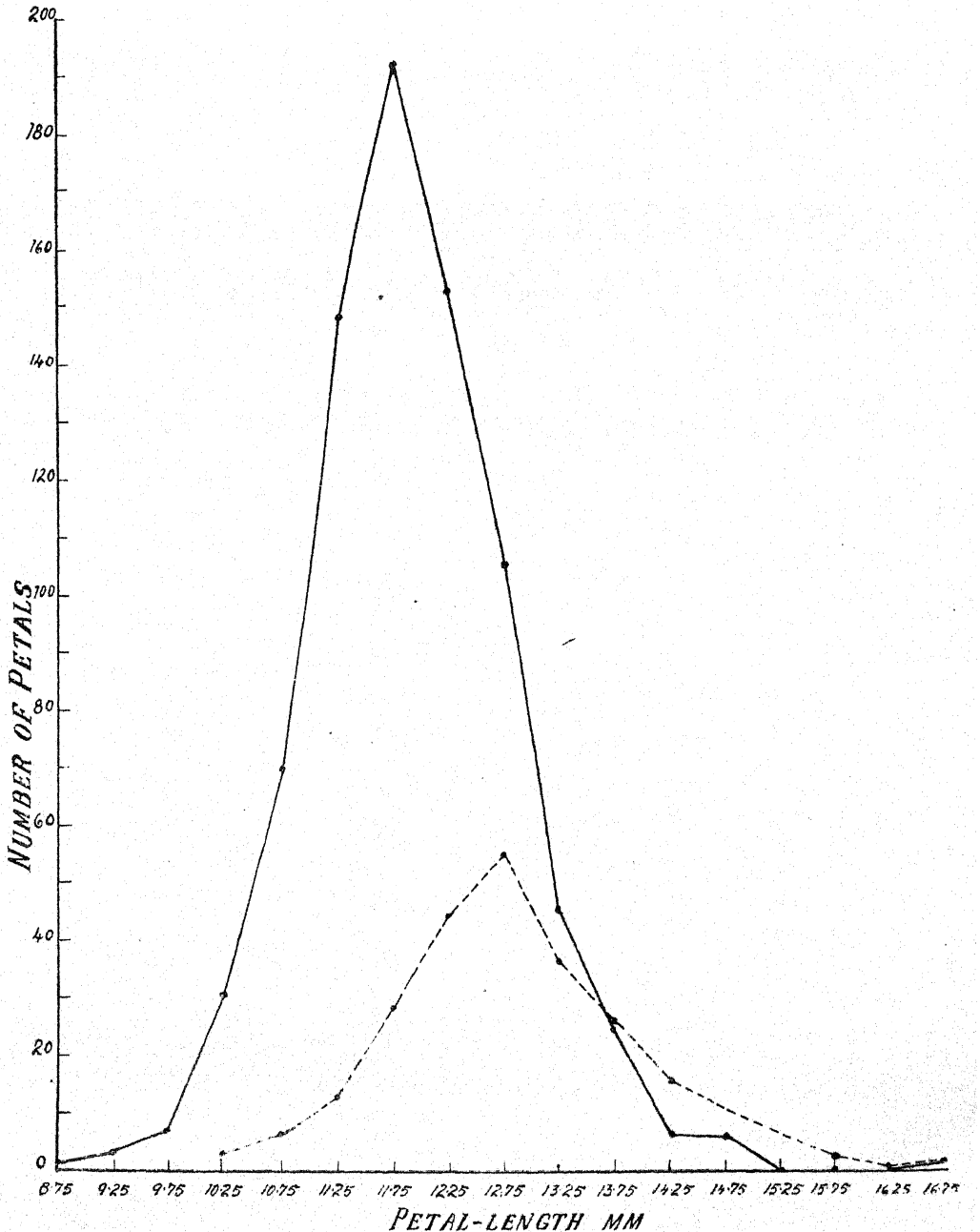


Fig. 6. Frequency curves for purple and non-purple petals in F_2.
Continuous line : purple petals.
Broken line : non-purple petals.

Pedicel-length.

The pedicel of Type 3 is short and that of Type 29 is long. The mean pedicel-length in Type 3 for 1928 was 11·07±0·16 mm. and for 1929 was 12·86±0·12 mm. and that in Type 29 for these two years was 24·20±0·12 mm. and 22·42±0·26 mm. respectively. The difference in the means for these two years in Type 3 is 8·9 times its probable error while that in the case of Type 29 is 6·3 times. These differences, therefore, are big enough to be statistically significant. The causes to which we can attribute these differences are again presumably the same as assumed in the case of differences in petal-length.

The F_1 pedicel was short with a mean length of 12·41±0·28 mm. which suggests dominance of short pedicel over the long.

The mean pedicel-length of F_2 was 14·85±0·054 mm. that is, a little higher than the F_1 mean. Both the standard deviation and the co-efficient of variation were fairly high in F_2. The range failed to reach the upper extremity of the long-pedicelled parent.

In F_3 some of the cultures with short pedicel bred true, while others gave a higher mean value. Cultures with long pedicel gave invariably a much lower mean. The variability in most of the cultures was lower than F_2.

The F_2 frequency curve (Fig. 7), it will be seen, is skew or lopsided. This is because there is preponderance of individuals in the ranges of Type 3 and F_1, which are overlapping, which corroborates the dominance of short pedicel over the long as indicated by F_1. It will be seen both from the curves (Fig. 7) and Table XI that the class 17 mm. is represented by Type 29, Type 3 and F_1 and on this ground if the F_2 range be dissected into two groups at this point the lower representing the

short pedicel and the upper the long, and the frequency 204 in this class, be added half and half to each of them, the situation becomes as shown below :—

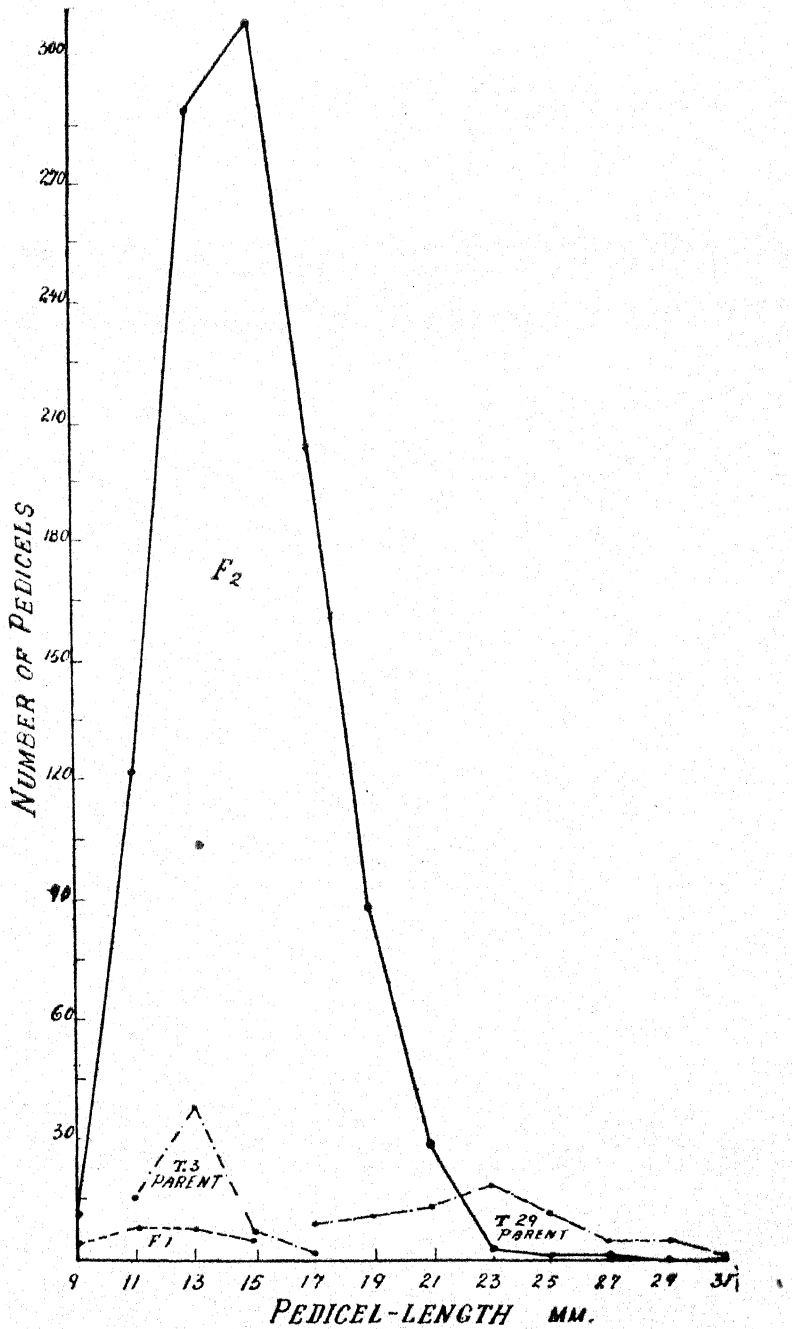


Fig. 7.—Frequency curves for pedicel-length.

	Overlapping ranges of Type 3 and F_1	Range of Type 29	
	11 122 287 310 204 89 28 2 1 1		=1055
	102	102	
Observed frequencies in the overlapping ranges of Type 3 and F_1, and Type 29	832	223	=1055
Expected frequencies on 3 : 1 basis	791·25	263·75	=1055

$$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{40{\cdot}75}{9{\cdot}46} = 4{\cdot}30$$

The fit is not good. However, considering that we are here dealing with a quantitative character it would not be wrong to conclude that the above results indicate a segregation on a 3 : 1 ratio.

The conclusions are further substantiated by the fact that the pedicel-length is linked with the colour in the plant, the short pedicel with purple colour and the long with non-purple. This is clearly brought out in Table XII in which the frequencies of purple and non-purple individuals together with their ratio as observed in each of the classes for pedicel-length are shown. It will be seen that the proportion of purple pedicels to non-purple increases as we go down from 14·01 to 16·00 mm. class towards the lower extremity until the lowest class is reached where no non-purple pedicel is present. Similarly as we go up from the class 16·01 to 18·00 mm. towards the upper extremity the proportion of non-purple pedicels to purple increases till the last two extreme classes are reached in which no non-purple individuals are represented, whereas, theoretically in each class the ratio of purples to non-purples should be 3 : 1.

TABLE XI.

Frequency distribution of length of pedicels, Type 3 × Type 29.

No.	Parental value	CLASS CENTRES IN MILLIMETERS 9	11	13	15	17	19	21	23	25	27	29	31	Total No. of pedicels	Mean	Standard deviation	Co-efficient of variation
Type 3 parent, 1928-29 . .	..	5	17	6	..	..	..	..	..	..	..	..	..	28	11·07 ± ·16	1·26 ± ·11	11·38 ± 1·03
,, ,, ,, 1929-30 . .	..	..	15	38	7	2	..	..	..	..	..	..	..	62	12·86 ± ·12	1·39 ± ·08	10·81 ± ·65
Type 29 parent, 1928-29 . .	..	..	..	..	..	..	..	4	10	11	4	1	..	30	24·20 ± ·12	1·97 ± ·17	8·18 ± ·71
,, ,, ,, 1929-30 . .	..	..	..	..	..	9	11	14	18	12	5	5	1	75	22·42 ± ·26	3·45 ± ·18	15·38 ± ·86
T. 3 × T. 29 F_1, 1928-29 . .	..	4	8	8	6	1	..	..	..	..	..	..	..	27	12·41 ± ·233	2·18 ± ·20	17·58 ± 1·66
,, ,, F_2, 1929-30 .	..	11	122	287	310	204	89	28	2	1	1	..	..	1055	14·85 ± ·054	2·59 ± ·038	17·44 ± ·26
,, ,, F_2, 1930-31 .	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
23—56 . . .	10·58	1	5	10	6	1	..	..	..	..	..	..	..	23	13·04 ± ·026	1·83 ± ·18	14·03 ± 1·41
8—86 . . .	10·74	..	3	8	19	12	7	3	0	1	..	..	..	53	15·98 ± ·25	2·74 ± ·18	17·15 ± 1·16
25—6 . . .	12·22	..	2	10	16	4	3	0	1	..	..	..	..	36	15·11 ± ·27	2·37 ± ·19	15·69 ± 1·26
5—18 . . .	14·40	1	6	7	9	4	1	..	..	..	..	..	..	28	13·86 ± ·30	2·34 ± ·21	16·88 ± 1·56
13—42 . . .	15·10	..	18	20	16	2	1	..	..	..	..	..	..	57	13·18 ± ·17	1·88 ± ·12	14·27 ± ·91
13—71 . . .	15·50	..	3	9	14	23	8	3	..	..	..	..	..	60	16·10 ± ·21	2·38 ± ·15	14·78 ± ·93
5—8 . . .	18·60	..	..	8	4	4	0	1	..	..	..	..	..	17	14·88 ± ·36	2·21 ± ·26	14·87 ± 1·76
8—83 . . .	20·60	..	..	3	9	8	3	5	1	1	..	..	..	30	17·33 ± ·38	3·06 ± ·27	17·65 ± 1·58
3—8 . . .	20·80	..	1	2	4	6	4	6	4	2	..	..	..	29	18·72 ± ·45	3·63 ± ·32	19·39 ± 1·78
8—24 . . .	23·46	..	..	..	5	4	10	1	1	..	..	..	..	21	17·95 ± ·31	2·10 ± ·22	11·69 ± 1·23

TABLE XII.

Frequencies of purple and non-purple pedicels in F_2 and their ratio in each class for pedicel-length.

Class centres in mm.	9	11	13	15	17	19	21	23	25	27	Total
Frequencies of purple and non-purple pedicels	Pr. N-pr. 9 0	Pr. N-pr. 107 12	Pr. N-pr. 242 41	Pr. N-pr. 248 63	Pr. N-pr. 129 73	Pr. N-pr. 48 41	Pr. N-pr. 11 16	Pr. N-pr. 1 2	Pr. N-pr. 0 1	Pr. N-pr. 0 1	Pr. N-pr. 795 250
Ratio of purple to non-purple pedicels	9 : 0	8·94 : 1	5·90 : 1	3·94 : 1	1·77 : 1	1·17 : 1	1 : 1·46	1 : 2	0 : 1	0 : 1	3·18 : 1

The F_2 frequencies of both the purple and non-purple individuals have been plotted (Fig. 8) from Table XII. It will be seen from the curves that both the curve for non-purple individuals and their modal class have

shifted up, which is an indication of longer pedicel while in absence of any linkage the two curves and the modal classes should be in very close agreement.

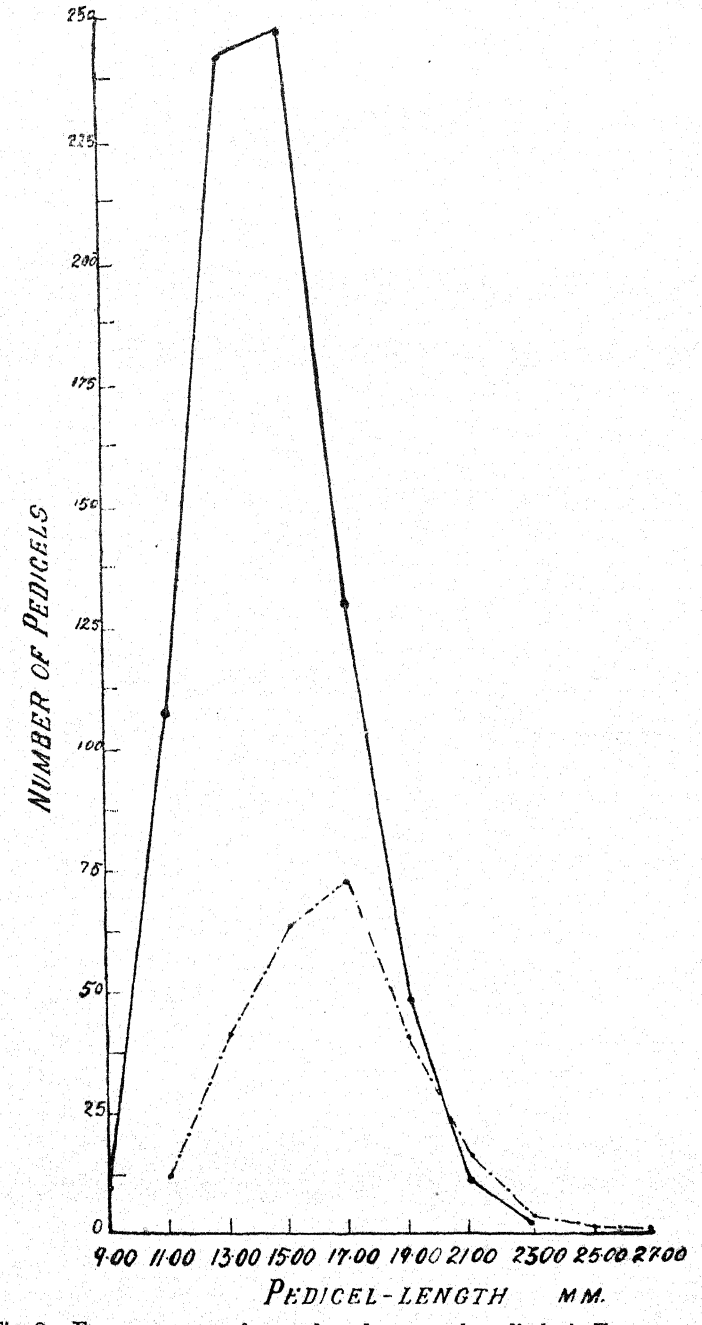


Fig. 8.—Frequency curves for purple and non-purple pedicels in F_2.
Continuous line : purple pedicels.
Broken line : non-purple pedicels.

If all the F_2 individuals be grouped into two classes, representing respectively the short and the long pedicel, the former measuring 17·00 mm. and below and the latter 17·01 mm. and above and the frequencies of purples and non-purples be found out in each, the following results are obtained :—

—	Short, purple pedicel	Short, non-purple pedicel	Long, purple pedicel	Long, non-purple pedicel	
Total observed	696	160	108	95	=1059
„ expected on 9 : 3 : 3 : 1 basis .	596·77	198·57	198·57	66·19	=1060·10
Deviation	+99·23	—38·57	—90·57	+28·81	

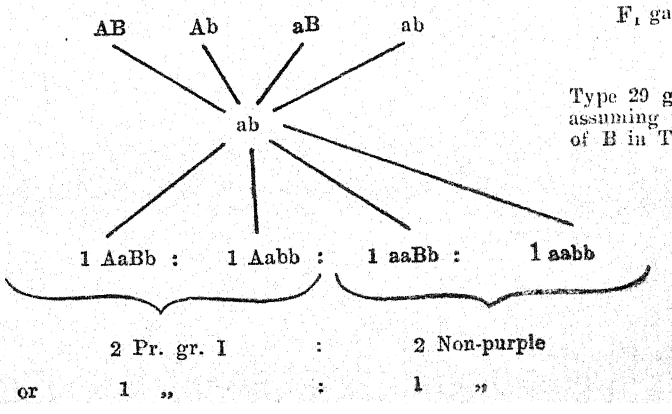
The excess in parental combinations suggests linkage. The cross-over value by the ' Product Ratio ' method comes to 33 per cent. which indicates significant linkage.

VI. The back-cross generation.

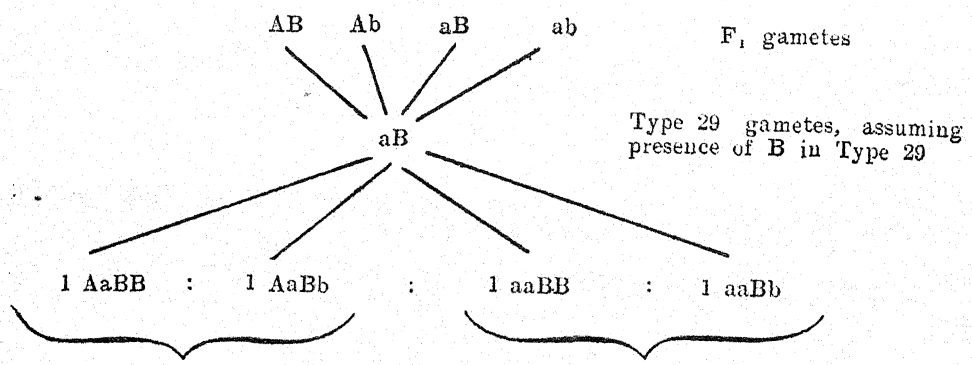
It was observed in F_2 that the phenotype Purple grade III was a shade deeper in purple colour than Type 3 and that the phenotype Purple grade II was less purple than the latter. However, Type 3 resembled Purple grade III more than Purple grade II.

The proof that the phenotype Purple grade III was genetically the same as Type 3 demanded evidence that the intensifying factor B was present in Type 3 itself-and not in Type 29. In order to ascertain this the F_1 was crossed with either parent. The cross of F_1 with Type 29, assuming either presence or absence of B in Type 29, did not help to clear the situation as on either assumption, in the back-cross generation, there should be only two phenotypes, Purple grade I and non-purple, in the ratio of 1 : 1 as shown below.

F_1 × Type 29



F_1 × Type 29



The actual results obtained are given below :—

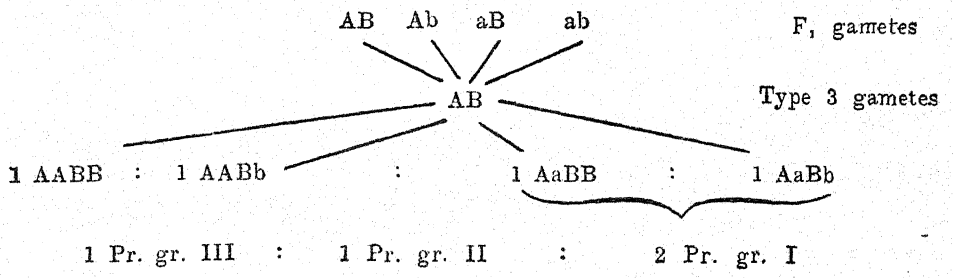
	Purple grade I	Non-purple	
Total observed	64	71	=135
„ expected on 1 : 1 basis	67·5	67·5	=135
Ratio observed	0·95 :	1·05	
„ expected	1 :	1	

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{3{\cdot}50}{3{\cdot}92} = 0{\cdot}89$. The fit is good.

It will be seen from the above that this back-cross does not throw any light on the genetical constitution of Type 29 so far as the intensifying factor B is concerned.

However, the cross of F_1 with Type 3 revealed the presence of B in Type 3 itself.

Assuming that B is present in Type 3 the following phenotypes and their proportions should be obtained in the back-cross generation.



The actual results obtained are as follows :—

—	Pr. gr. III	Pr. gr. II	Pr. gr. I	—
Total observed	5	4	9	=18
„ expected on 1 : 1 : 2 basis . .	4·5	4·5	9	=18
Ratio observed	1·11 :	0·89 :	2	
„ expected	1 :	1 :	2	

The realization of this result is only possible if B, the intensifying factor, be present in Type 3. Though the population is small the results are convincing.

The other object of crossing F_1 back to Type 29 was to ascertain if the factors for colour in plant and colour in ripe fruit in Type 3 × Type 29 were really linked as indicated by the F_2 and F_3 results and if so in what proportion were the F_1 gametes produced.

If each character be taken separately a good 1 :1 ratio is obtained in the back-cross as is shown below :—

—	Purple plants	Non-purple plants	—
Total observed	44	50	=94
„ expected on 1 : 1 basis	47	47	=94

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{3}{3{\cdot}26} = 0{\cdot}92$. The fit is very good.

—	Red fruits	Orange fruits	—
Total observed	42	52	=94
„ expected on 1 : 1 basis	47	47	=94

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{5}{3{\cdot}26} = 1{\cdot}53$. The fit, therefore, is good.

If the two characters are taken together the four phenotypes do not occur in equal numbers, the frequency of the combined parental phenotypes being 1·47 times that of the other two phenotypes. The results are :—

—	Purple gr. I, red fruit	Purple gr. I, orange fruit	Non-purple, red fruit	Non-purple, orange fruit	—
Total observed . . .	24	20	18	32	=94
„ expected on 44 per cent. cross-over value	26·32	20·68	20·68	26·32	=94
Deviation	2·32	0·68	2·68	5·68	
Ratio observed	1·16 :	0·97 :	0·87 :	1·54	
„ expected on 44 per cent. cross-over value	1·3 :	1 :	1 :	1·3	

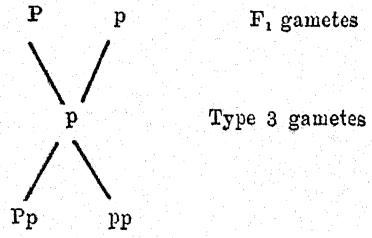
$\chi^2 = 1{\cdot}80$; $P = 0{\cdot}618$.
The fit, therefore, is good.

On a basis of 44 per cent. cross-over we obtain a value of χ^2 of 1·80 whereas assuming that there is no linkage a value of χ^2=4·89 is obtained. The evidence is, therefore, in favour of weak linkage. The frequencies in the reciprocal, however, gave no indication of linkage and indeed a cross-over value of 44 per cent. approximates very closely to independent segregation.

The back-crosses have further confirmed that the fruit-position (pendent or erect) and colour of ripe fruit (red or orange) are each inherited on a 3 : 1 monohybrid ratio.

As regards the former character there should be on theory only two phenotypes, pendent and erect, in the proportion of 1 : 1 in the back-cross generation as shown below :—

F_1 × Type 3 parent



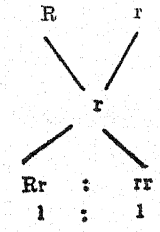
The actual results obtained are given below :—

	Pendent-fruited plants	Erect-fruited plants	
Total observed	10	8	=18
„ expected on 1 : 1 ratio	9	9	=18

$\frac{\text{Deviation}}{\text{Probable Error}} = \frac{1}{2{\cdot}86} = 0{\cdot}35$. The fit is good.

As regards the colour of ripe fruit we should obtain on theory red and orange phenotypes in the proportion of 1 : 1 in the back-cross generation as shown below :—

F_1 × Type 29 parent



F_1 gametes

Type 29 gametes

i.e., 1 red : 1 orange.

The actual results obtained which agree with the theory are already given on page 286.

VII. Theory.

From the results of the cross and the back-crosses it is assumed that the following factors determine the various qualitative characters studied in this cross.

A—a factor which produces purple colour in all plant organs. If it is absent, the stem, leaf and fruit remain green, petals and filaments white, style pale purple or white, stigma yellow, and anthers bluish-yellow.

B—a factor which in the presence of A intensifies purple colour but is inert by itself.

Both A and B when homozygous have double the effect to when heterozygous; but the effect of two doses of B is less than one of A.

P—a factor which causes the fruit to be pendent, in absence of which, the fruit remains erect.

R—a factor which produces redness in ripe fruit and seed, in absence of which, these remain yellow or orange.

D—a factor which causes the fruit-apex to be blunt. When D is heterozygous the apex is partially pointed. If D is absent the apex remains pointed.

F—a factor which causes the bulging of the fruit-base. If F is absent the base remains unbulged.

E—a factor which prevents the calyx from enclosing the fruit-base. When E is absent the base remains enclosed.

On the basis of the functions performed by each of the above factors and from the results of the cross and the back-crosses the genetic constitution of the parents and the F_1 should be as follows:—

Type 3—AABBppRRDDFFEE.

Type 29—aabbPPrrddffee.

F_1—AaBbPpRrDdFfEe.

VIII. Application of theory to the observed results.

It will now be seen how far the theory agrees with the results obtained in F_2 and F_3.

In the first character that is studied, *viz.*, the purple colour in the plant, both A and B will be segregating. The F_1 should, therefore, be heterozygous for both these and hence should appear intermediate in purple colour and the F_2 should show a population of the following phenotypic proportions on dihybrid basis.

AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb
1	2	1	2	4	2	1	2	1
Pr. gr. III like Type 3	Pr. gr. II	Pr. gr. II	Pr. gr. I	Pr. gr. I	Pr. gr. I	Non-purples		
	Less purple than Type 3		Like F_1			Like Type 29		

1 : 3 : 8 : 4

or 12 purples of all grades : 4 non-purples

or 3 : 1

This is closely realized in F_2 (pages 230, 231 and 232).

The phenotype Purple grade III, which has only one genotype, AABB, should be purple like Type 3 parent. The individuals of this class should be one-sixteenth of the entire F_2 population and should breed true in F_3 as is realized (pages 231 and 232).

The phenotype Purple grade II, which contains the genotypes AABb and AAbb should be less purple than Purple grade III as the first contains only one intensifying dose while the second is without either. The former should be a shade deeper than the latter. Actually it was not possible to separate these two genotypes from one another with certainty as the colour differences were not sharp. The fact is that the colour even in a single individual plant showed a good amount of variation depending upon the age of its various organs and the amount of illumination which they received.

On theory, then, this phenotype, which has been termed as Purple grade II, should be three times the number of plants like Type 3 in colour or three-sixteenth of the total F_2 population as is realized (pages 230 and 231) and should behave in F_3 as follows :—

One-third of these Purple grade II cultures with the genetic constitution AAbb should breed true and the remaining two-thirds with the genetic constitution AABb should segregate into Purple grades III and II, as B is heterozygous, in the ratio of 1 Purple grade III : 3 Purple grade II, as shown below :—

AABB		AABb		AAbb
Pr. gr. III		Pr. gr. II		Pr. gr. II
1	:	2	:	1
		3		
1	:			

This is realized (pages 231 and 232).

The phenotype Purple grade I (like F_1) which consists of the genotypes AaBB, AaBb, and Aabb should be much less purple than the Purple grade II individuals as it is heterozygous for A, the basic colour factor itself. Amongst the genotypes themselves the first should be the most purple, the second a little less and just like the F_1, and the third the least. Here again as in the case of Purple grade II and for the same reasons it was not possible to separate out the genotypes with certainty and they were kept together as individuals "like F_1" in one class which has been called Purple grade I.

Theoretically these individuals should be fifty per cent. of the entire F_2 population as is realized (pages 230 and 231) and this phenotype should behave in F_3 as follows :—

Those with the genetic constitution AaBB should be one-fourth of all the Purple grade I individuals in F_2 and should segregate in F_3, as A is heterozygous, into Purple grade III, Purple grade I, and non-purples as shown below :—

AABB		AaBB		aaBB
Pr. gr. III		Pr. gr. I		Non-pr.
1	:	2	:	1

The number of Purple grade I cultures segregating in this fashion in F_3 should be one-fourth of the number in that class. The expectations are realized in F_3 (pages 231 and 232).

The next genotype, AaBb, which is the same as the F_1 in genetical constitution, should theoretically be half of the total number of F_2 individuals of Purple grade I and should segregate in F_3 like F_2 as both A and B are heterozygous. The number of Purple grade I cultures in F_3, segregating like F_2, should be half the total number of such cultures. The F_3 behaviour of these cultures should be like F_2 as follows :—

	1 AABB	2 AABb 1 AAbb	2 AaBB 4 AaBb 2 Aabb	1 aaBB 2 aaBb 1 aabb
	Pr. gr. III	Pr. gr. II	Pr. gr. I	Non-purple
	1	: 3	: 8	: 4
or		Purples of all grades		Non-purples
		12	:	4
or		3	:	1

The F_3 results are in agreement with the theory (pages 231 and 232).

The third and the last genotype, Purple grade I, which has Aabb as its genetic formula, should be one-fourth of the total number of F_2 individuals belonging to this class and should segregate in F_3 into Purple grade II, Purple grade I, and Non-purples in the following ratio :—

1 AAbb	:	2 Aabb	:	1 aabb
Pr. gr. II		Pr. gr. I		Non-purple.

The number of F_2 cultures segregating as above in F_3 should be one-fourth of the total number of cultures of Purple grade I. The expectations are realized in F_3 (pages 231 and 232).

The non-purple phenotype, which consists of the genotypes, aaBB, aaBb, and aabb in the proportion of 1 : 2 : 1 respectively, should form one-fourth of the total F_2 population and breed true in F_3 as A, the colour factor, is absent irrespective of the presence or absence of B, the colour intensifying factor. This is realized in F_2 and F_3 (pages 230 and 232 ; 231 and 232).

With regard to fruit-position, the F_1 should be heterozygous for P and be pendent-fruited and should segregate in F_2 into pendent and erect fruited plants on a 3 : 1 basis as follows :—

PP		Pp		pp
1	:	2	:	1
3 pendent			:	1 erect

In F_3, one-third of the cultures from pendent-fruited plants should breed true for pendent character as they are expected to be homozygous for P and two-thirds, which are expected to be heterozygous for this factor, should segregate again like F_2 in a 3 pendent : 1 erect monohybrid ratio.

The expectations have been realized in F_2 and F_3 (pages 233, 234, 235), except that the deviations between theoretical and observed numbers in the case of homozygous dominant and heterozygous cultures in F_3 of Type 3 × Type 29 were rather high. Also some of the plants in F_1, F_2 and F_3 had a few of their early fruits erect or intermediate. Some of these F_2 plants were grown in F_3 and were all found to be heterozygous for P. On theory, however, P, even if heterozygous should be perfectly dominant and should not produce any erect fruits. The results, then, are somewhat contradictory to the theory. The proportion of these plants, showing both erect and pendent fruits to those showing only pendent forms, is very small indeed in F_2 though much higher in F_1 (Tables III and IV).

With regard to the colour in the ripe fruit, red or orange, the F_1 should be heterozygous for R and should be red. The F_2 population should consist of the following phenotypic proportions :—

1 RR	:	2 Rr	:	1 rr
3 red-fruited			:	1 orange-fruited

In F_3, one-third of the cultures grown from red-fruited F_2 plants should breed true as they are expected to be homozygous for R and two-thirds should segregate like F_2 on a 3 : 1 monohybrid basis as they are expected to be heterozygous for this factor. These results have been realized in F_2 and F_3 (pages 236 and 237).

If the purplish tinge in the fruit be also considered with the colour of ripe fruits there is then to be considered a segregation of three factors, *viz.*, A, B, and R.

The F_1 is AaBbRr, *i.e.*, purplish red, and is easily distinguished from the deeper purplish red colour of Type 3. The proportion of F_2 phenotypes should be as follows :—

27 ABR 9 AbR	9 aBR 3 abR	9 ABr 3 Abr	3 aBr 1 abr
36 purplish red or 9 „ „	: 12 red 3 „	: 12 purplish orange 3 „ „	: 4 orange 1 „

This is realized to some extent in F_2 (page 251) but the fit was not good due to discrepancies in the parental classes, suggesting linkage between A and R. As this linkage has been dealt with already in another chapter it is unnecessary to go into it here any further.

With regard to fruit-apex, as D is segregating the F_1 should be heterozygous for D and should be partially pointed and the F_2 segregation should be on a monohybrid basis as shown below :—

DD		Dd		dd
1	:	2	:	1
1	:	3		
Blunt		Partially pointed and pointed		
like Type 3		like F_1 and Type 29		

This result is realized in F_2 (pages 238, 239) and confirmed by F_3 (page 239), except that 1 culture, recorded as blunt-fruited in Type 3 × Type 29 segregated like F_2 while on theory all blunt cultures should be pure. The diagnosis in F_2 appears to have been wrong in this case.

As regards bulging or non-bulging of fruit-base there is segregation of factor F and the F_1 should be heterozygous for F and should appear bulged. In F_2 the segregation should be on a 3 bulged : 1 non-bulged monohybrid basis as shown below :—

FF		Ff		ff
1	:	2	:	1
3			:	1
Bulged				Non-bulged

The expectation is realized in F_2 and confirmed by F_3 (pages 240, 241).

In the behaviour of the calyx—whether enclosing fruit-base or not—there is segregation of E. The F_1 should be heterozygous for E and should have an unenclosed fruit-base. The F_2 should show the following monohybrid segregation :—

EE		Ee		ee
1	:	2	:	1
3			:	1
Fruit-base not enclosed				Fruit-base enclosed

This is realized in F_2 and confirmed by F_3 (pages 242-3).

In considering the inheritance of purple colour in plant and fruit-position together we are dealing with a segregation of the factors A, B, and P. The F_1 should be heterozygous for these and in F_2 we should expect a population consisting of the following phenotypic proportion :—

3 AABBP	1 AABBp	6 AABbP	2 AABbp	6 AaBBP	2 AaBBp	3 aaBBP	1 aaBBp
		3 AABbp	1 AAbbp	12 AaBbP	4 AaBbp	6 aaBbP	2 aaBbp
				6 AabbP	2 Aabbp	3 aabbP	1 aabbp
3 Pr. gr. III pendent	1 Pr. gr. III erect	9 Pr. gr. II pendent	3 Pr. gr. II erect	24 Pr. gr. I pendent	8 Pr. gr. I erect	12 Non-pr. pendent	4 Non-pr. erect
Or	27 ABP	9 ABp		9 aBP	3 aBp		
	9 AbP	3 Abp		3 abP	1 abp		
	36 purple pendent	12 purple erect		12 Non-purple pendent	4 Non-purple erect		
Or	9 „ :	3 „ :		3 „ :	1 „		

This result has been closely realized in F_2 and confirmed by F_3 (pages 244-50).

With regard to the inheritance of the two characters, purple colour and colour of ripe fruit, when considered together we have A, B and R segregating. The F_1 should hence be heterozygous for all the three factors and the following phenotypic proportions should be expected in F_2 :—

3 AABBR	1 AABBr	6 AABbR	2 AABbR	6 AaBBR	2 AaBBr	3 aaBBR	1 aaBBr
		3 AAbbR	1 AABbr	12 AaBbR	4 AaBbr	6 aaBbR	2 aaBbr
				6 AabbR	2 Aabbr	3 aabbR	1 aabbr
3 Pr. gr. III red	1 Pr. gr. III orange	9 Pr. gr. II red	3 Pr gr. II orange	24 Pr. gr. I red	8 Pr. gr. I orange	12 Non-pr. red	4 Non-pr. orange
Or	27 ABR	9 ABr		9 aBR	3 aBr		
	9 AbR	3 Abr		3 abR	1 abr		
	36 purple red	12 purple orange		12 non-purple red	4 non-purple orange		
Or	9 „ :	3 „ :		3 „ :	1 „		

The results which we have actually obtained in F_2 of Type 3 × Type 29 (page 251) show that the parental characters—"purple colour" and "red fruit" and "non-purple colour" and "orange fruit" keep together more often than would be expected on the basis of independent segregation. This suggests that the two factors A and R are linked, and the F_1 gametes, (so far as these two factors are concerned), AR, Ar, aR and ar, instead of being produced in equal numbers should be approximately produced in the following proportions for a cross-over value of 44 per cent. :—

AR		Ar		aR		ar
1·3	:	1	:	1	:	1·3

The back-cross, F_1 × Type 29, actually produced the phenotypes in the following proportions :—

1·16	:	0·97	:	0·87	:	1·54

These results are in fair agreement with what would be expected for a 44 per cent. cross-over value.

In dealing with fruit-position (pendent or erect) and colour of ripe fruit together we have a segregation of P and R. The F_1 should be heterozygous for these factors. In F_2 a population consisting of the following phenotypic proportions would be expected :—

PR		Pr		pR		pr
9		3		3		1
9 pendent, red fruit	:	3 pendent, orange fruit	:	3 erect, red fruit	:	1 erect, orange fruit

Expected results have been realized in F_2 and F_3 (pages 257-8).

Considering the three characters, purple colour, fruit-position, and colour of ripe fruit together we have A, B, P, and R segregating. The F_1 would be heterozygous for all these and in F_2 we should expect to get a population of the following phenotypic proportions :—

	9 AABBPR	3 AABBPr	3 AABBpR	1 AABBpr	18 AABbPR 9 AAbbPR	6 AABbPr 3 AAbbPr	6 AABbpR 3 AAbbpR	2 AABbpr 1 AAbbpr	
	9 Pr. gr. III, pendent, red	3 Pr. gr. III, pendent, orange	3 Pr. gr. III, erect, red	1 Pr. gr. III, erect, orange	27 Pr. gr. II, pendent, red	9 Pr. gr. II, pendent, orange	9 Pr. gr. II, erect, red	3 Pr. gr. II, erect, orange	
	18 AaBBPR	6 AaBBPr	6 AaBBpR	2 AaBBpr	9 aaBBPR	3 aaBBPr	3 aaBBpR	1 aaBBpr	Summating genotypes
	36 AaBbPR	12 AaBbPr	12 AaBbpR	4 AaBbpr	18 aaBbPR	6 aaBbPr	6 aaBbpR	2 aaBbpr	
	18 AabbPR	6 AabbPr	6 AabbpR	2 Aabbpr	9 aabbPR	3 aabbPr	3 aabbpR	1 aabbpr	
which belong to the same phenotype	72 Pr. gr. I, pendent, red	24 Pr. gr. I, pendent, orange	24 Pr. gr. I, erect, red	8 Pr. gr. I, erect, orange	36 non- pr., pendent, red	12 non- pr., pendent, orange	12 non- pr., erect, red	4 non- pr., erect, orange	
or	81 ABPR	27 ABPr	27 ABpR	9 ABpr	27 aBPR	9 aBPr	9 aBpR	3 aBpr	
	27 AbPR	9 AbPr	9 AbpR	3 Abpr	9 abPR	3 abPr	3 abpR	1 abpr	
	108 purple, pendent, red	36 purple, pendent, orange	36 purple, erect, red	12 purple, erect, orange	36 non- purple, pendent, red	12 non- purple, pendent, orange	12 non- purple, erect, red	4 non- purple, erect, orange	
or	27 purple pendent, red	9 purple, pendent, orange	9 purple, erect, red	3 purple, erect, orange	9 non- purple, pendent, red	3 non- purple, pendent, orange	3 non- purple, erect, red	1 non- purple, erect, orange	

Due to the linkage between A and R, which we have already considered, there are discrepancies between the observed results and the theoretical expectations (page 295).

IX. Discussion.

Purple colour in the plant of Type 3 according to our investigations seems to depend upon two factors, one of which is a basal colour factor, which we have called A, and the other, an intensifying factor, which we have designated as B. The latter, besides intensifying purple colour, has no other function to perform. The appearance of slightly deeper purple plants than Type 3 in F_2 suggested that the intensifying factor B might be contributed by Type 29 but on the evidence of the results obtained by crossing F_1 back to the Type 3 it is certain that both A and B are present in Type 3 itself.

The whole purple population in F_2 was grouped into classes, Purple grade III (like Type 3), Purple grade II (less purple than Type 3), and Purple grade I (like F_1), although indications of genetical variations in the last two classes were not wanting. The idea to sub-divide these two classes into still further grades of purple had to be given up due to the fact that the environment plays a considerable part in determining the intensity and extent of colour in plant and thus renders further classification difficult.

Regarding the colour in the style of Type 29 we can only say definitely that it is quite distinct from the Type 3 colour and is completely linked with the non-purple colour in the plant. Due to the considerable amount of variation that it has shown even in the same plant, it has not been possible for us to ascertain how this purple colour in the style of Type 29 is inherited.

Regarding the fruit-position, the appearance of both the allelomorphs, pendent and erect fruit-positions on one and the same individual cannot be properly explained. It was thought that this might be a case of somatic segregation but the segregation of both the pendent and the erect fruits on the same plant on a 3 pendent : 1 erect basis in the next generation proved that this was not so. Had this been a case of a somatic segregation all the erect and some of the pendent fruits should have bred true to their respective positions.

This aberrant condition does not either seem to be due entirely to heterosis as all the F_1 plants did not show it.

As this variation is observed in F_1 of both the cross and its reciprocal, it does not look likely that this is due to prepotency of either parent as male or female.

Ikeno [1928] has suggested that this is due to influences of the environment, such as differences in temperatures, but we are in a position to say that this does not seem to be the cause of variation in our case at least as two F_1 plants which were kept over and which passed through summer, monsoons, and the winter of one year, produced only pendent fruits. Besides, the fact that such aberrations have, in our case, invariably occurred only at the beginning of the first fruiting suggests

that the cause is not in any environmental stimulus but is some kind of activity or forces within the plant between the two allelomorphic characters just at this time such that each tries to dominate over the other with the result that the recessive character gets sometimes a chance to show itself before the dominant character establishes its dominance. The fact, that the percentage of such aberrant plants is far greater in F_1 than in F_2, further shows that this especially happens in plants which are most heterozygous.

Whatever be the cause of this abnormality it (the abnormality) is helpful in knowing the constitution of a plant for this character beforehand. If this phenomenon were to occur in a hybrid population regularly, one could distinguish with certainty the heterozygous pendents from the homozygous a year beforehand as this occurs, so far as our observations are concerned, invariably in an individual which is heterozygous for this character.

The colour in ripe fruit is influenced by a single factor but the presence of A (purple colour) in an individual gives the fruit a purplish tinge. Thus instead of two phenotypes, "red" and "orange", in F_2, for colour of ripe fruit, we have really speaking four phenotypes, *viz.*, "purplish red", "red", "purplish orange" and "orange". In other words, we have to consider here a segregation of both A and R on a 9 : 3 : 3 : 1 basis instead of the segregation of R alone on a monohybrid basis.

In dealing with "fruit-base" and "calyx enclosing fruit-base" or "not enclosing fruit-base" we have two characters showing so much interdependence on one another. Excepting a few cases we found in F_2 that a bulged fruit-base was nearly always unenclosed by the calyx and that an unenclosed, unbulged fruit-base was very rarely met with. We have calculated the actual linkage intensity between these two characters by the 'Product Ratio' method which gives a cross-over value of 3 per cent.

In length of pedicel we have a case where the smaller pedicel is dominant over the longer. Such cases are somewhat uncommon.

In dealing with the quantitative characters we have not been able to show definitely the nature of inheritance in each. We have discovered that all the size characters, which we have studied, are to a more or less degree linked with one or more of the qualitative characters, *viz.*, colour in plant, fruit-position, and calyx behaviour segregating on a 3 : 1 ratio.

The linkage relations between the qualitative and the quantitative characters go to suggest that the latter are inherited on very much the same basis as the former. In our opinion, the mode of inheritance in the quantitative characters is as simple as in the qualitative ones, but what makes it look complicated is the environment. Thus it will be seen that in our observations in "length of petal"

and "length of pedicel" in the pure parental types (pages 273, 278) we have found that the mean lengths for 1928 and 1929 differed so much as to be statistically significant. This may be rather unusual but the fact that environments considerably influence size characters remains.

Our observations so far as the Type 3 × Type 29 cross is concerned show that petal-length, pedicel-length, and fruit-length are linked with the colour in plant which is in its turn linked with the colour of ripe fruit. We have enough evidence to show also that the fruit-length is also linked with the fruit-position (Table VII). Thus we conclude that all the above characters, both qualitative and quantitative, have their genes located in one of the twelve chromosomes, which is the haploid number in this plant.

The absence of any indication of linkage between "colour in plant" and "colour of ripe fruit" in the reciprocal cross Type 29 × Type 3, is, in our opinion, due to the fact that the linkage intensity between these characters as observed in the cross, Type 3 × Type 29, is indeed very low (44 per cent. cross-over) and that we may have failed to realize it in a small F_2 population of the reciprocal.

X. Summary and conclusion.

The study of this cross has established that the purple colour in plant is due to two factors, one of which is a basal colour factor and the other which merely intensifies it. The cross of F_1 with Type 3 has proved that this latter is also present in Type 3. The purple colour is partially dominant to its allelomorph.

2. Fruit-position is inherited on a simple monohybrid basis, pendent condition being dominant to erect, but as shown by our observations variations in the fruit-position occur even in the same plant when the plant is heterozygous for this dharacter.

3. The inheritance of colour of ripe fruit is determined by a single factor, red being dominant to orange.

4. Weak linkage between colour in plant and colour of ripe fruit has been discovered in the cross Type 3 × Type 29 and confirmed by the back-cross results.

5. The inheritance of fruit-apex is determined by a single factor.

6. The nature of the fruit-base (bulging or not bulging) and that of the calyx (enclosing or not enclosing fruit-base) are found to be interdependent on one another and each is found to be inherited on a monohybrid basis. Linkage between these two characters is strong, the cross-over value being 3 per cent.

7. Except for the low linkage between colour in plant and colour of ripe fruit in Type 3× Type 29 the characters, *viz.*, colour in plant, fruit-position and colour of

ripe fruit, when taken in pairs or all the three together, have been found to segregate independently.

8. Length of fruit is probably inherited on a tri-hybrid basis in the ratio of 3 short and intermediate fruits to 1 long. This is to some extent corroborated by the fact that the fruit-length is linked with the colour in plant and fruit-position both of which segregate on a 3 : 1 basis.

9. Thickness of fruit is inherited on a simple monohybrid ratio. The linkage between fruit-thickness and calyx behaviour (enclosing or not enclosing fruit-base) has confirmed this.

10. The petal-length is also found to be linked with the colour in plant. The ratio of short and intermediate petals to the long ones is 3 : 1.

11. Short pedicel is found to be dominant over the long on a 3 : 1 basis. Pedicel length has been found to be linked with the colour in plant.

12. The F_1 manifested heterosis in the following characters :—vigour, maturity, height of plant, productivity, and thickness of fruit.

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THE INFLUENCE OF GREEN MANURE AND ORGANIC RESIDUES ON NITROGEN FIXATION IN SOIL.

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It has been known for many years that the addition of soluble carbohydrates, such as glucose, cane sugar, and mannite, in the proportion of one or two per cent. to soils is followed by measurable gains in soil nitrogen after two or three weeks' incubation, provided temperature and moisture conditions are favourable. When green manures and other plant residues are added to soil, these are broken down by the action of soil organisms into simpler carbohydrates, which might prove to be a source of energy for the nitrogen-fixing bacteria, and the beneficial effect of the organic manures might in some degree arise from the subsequent fixation of nitrogen. To find out if such fixation of nitrogen does actually take place, several series of experiments were carried out.

In the first series, whole green plant of *bajra* (*Pennisetum typhoideum*), maize (*Zea Mays*), cowpea (*Vigna catiang*), and *dhaincha* (*Sesbania aculeata*) including roots and the leaves of the *ashoka* tree (*Polyalthia longifolia*), were used as manure after being cut up as finely as possible with scissors. Samples were taken for the estimation of moisture and nitrogen, and the green stuff was added to Pusa soil in the proportion of two grms. per hundred grms. of soil. The moisture-content of the soil was made up to 16 per cent., the optimum moisture-content for Pusa soil, by the addition of distilled water.

A second series was started at the same time which differed from the first in that moisture was made up to 16 per cent. by the addition of extract of well rotted manure. This extract was prepared by shaking 10 grms. of manure in a litre of water, allowing it to settle, and filtering off the superanatant liquid. The extract added 1 mgm. nitrogen to the original content of 48 mgms. per 100 grms. soil. The second series was started because it was considered possible that the bacteria introduced by the manure extract might expedite the breaking down of the complex carbohydrates into simpler forms to be utilised by the nitrogen-fixing bacteria.

In the two series, duplicate lots of 600 grms. soil were taken for each material. Incubation was at 30° C. and the loss of moisture was made up once a week.

At the end of two months a large sample of about 100 grms. was withdrawn, air-dried, well mixed, seived, and nitrogen estimated by modified Kjeldahl methods in duplicate samples of 10 grms. each. $N/14$ acid and alkali were used for titrations. More dilute acid and alkali did not give a well-defined sharp end point. Methyl red was used as an indicator. Similar estimations of nitrogen were done after three months' incubation.

The results given in Tables I and II show that definite quantities of nitrogen were fixed in all cases and that these were greatly increased by the addition of manure extract to maize, *bajra* and cowpea. Though control series with soil and moisture alone were not started simultaneously, in several experiments carried out in another connection at the time, in which the Pusa soil alone was incubated with moisture, the nitrogen fixed was found to vary from 0·5 to 1·5 mgms. per 100 grms. of soil. The large increase of nitrogen in treated soil is therefore to be attributed to the addition of organic material.

TABLE I.

Nitrogen fixation per 100 grms. soil by addition of fresh green plant tissues.

(*Mgms. or nitrogen per 100 grms. of air-dry soil*)

Green plant	Nitrogen added as green manure	Total nitrogen at start	After 2 months				After 3 months			
			Total nitrogen	Increased nitrogen	Average	Increase per grm. added material on dry basis	Total nitrogen	Increased nitrogen	Average	Increase per grm. added material on dry basis
Bajra (moisture 88 per cent.)	6·35	54·35	58·5 56·5	4·15 2·15	3·15	13·12	59·5 58·5	5·15 4·15	4·65	18·6
Maize (moisture 88 per cent.)	6·02	54·02	57·0 58·5	2·98 4·48	3·73	15·62	58·0 59·5	3·97 5·47	4·72	18·9
Dhaincha (moisture 84 per cent.)	11·65	59·65	63·0 64·5	3·35 4·85	4·10	12·80	64·0 64·5	4·35 4·85	4·60	14·4
Cowpea (moisture 86 per cent.)	12·85	60·85	63·5 65·0	2·65 4·15	3·4	12·14	64·0 65·0	3·15 4·15	3·65	13·0
Ashoka leaves (moisture 66 per cent.)	18·72	66·72	69·5 69·0	2·78 2·28	2·5	3·75	69·5 70·0	2·77 3·27	3·0	4·5

TABLE II.

Effect of unfermented green manure supplemented with manure extract on nitrogen fixation in soil.

(*Mgms. of nitrogen per 100 grms. of air-dry soil*)

Treatment	Nitrogen added as green manure and manure extract	Total nitrogen at start	After 2 months				After 3 months			
			Average total nitrogen	Nitrogen fixed	Average nitrogen fixed	Nitrogen fixed for 1 grm. of dry green manure	Average total nitrogen	Nitrogen fixed	Average nitrogen fixed	Nitrogen fixed for 1 grm. of dry green manure
Bajra . . .	7·35	55·35	64·0	8·65	7·65	31·9	64·5	9·15	8·65	34·0
			62·0	6·65			63·5	8·15		
Maize . . .	7·02	55·02	61·0	5·98	5·73	22·8	61·5	6·48	7·03	28·0
			60·5	5·48			62·6	7·58		
Dhaincha . . .	12·65	60·65	64·0	3·35	2·85	9·2	65·0	4·35	3·85	12·0
			63·0	2·35			64·0	3·35		
Cowpea . . .	13·85	61·85	69·0	7·15	6·9	24·6	69·5	7·65	7·40	26·4
			68·5	6·65			69·0	7·15		
Ashoka leaves . .	19·72	67·72	68·5	0·78	1·78	2·7	71·5	3·78	3·04	4·5
			70·5	2·78			70·0	2·28		

In our next experiments we tried to find out whether rotting of the green stuff previous to its application to the soil had any effect, beneficial or otherwise, on the fixation of nitrogen.

The materials used were, as before, maize, cowpea and *dhaincha* plants and *ashoka* leaves, but sann hemp (*Crotalaria juncea*) was substituted for *bajra* of which further supply was not available.

They were chopped finely and packed in jars, with loosely fitting covers after withdrawing samples for the estimation of nitrogen and moisture.

Two jars of each material were taken, to one of which was added 5 per cent. of manure extract, made as previously described, and to the other 5 per cent. of distilled water.

The materials were stirred and mixed every week to ensure uniform fermentation. Samples were withdrawn at the end of one, two and three months, for the estimation of nitrogen and moisture, and from these figures, together with that of the loss in weight of the bulk, the total nitrogen remaining in the bulk was calculated, and the results are shown in Table III.

TABLE III.

Data about the fermentation of the green manures.

Green manure	Treatment	Moisture per cent.	Total nitrogen in 100 grms.	After 1 month			After 2 months			After 3 months		
				Moisture per cent.	Per cent. loss of weight	Total N in the fermented material 100 grms. of the unfermented manure	Moisture per cent.	Per cent. loss of weight	Total N in the fermented material 100 grms. of the unfermented manure	Moisture per cent.	Per cent. loss of weight	Total N in the fermented material 100 grms. of the unfermented manure
Maize	5 per cent. H_2O	88	300·0	92·0	18·0	282·0	90·0	10·0	272·0	87·5	9·0	261·4
Maize	5 per cent. M E	88	300·0	92·2	17·7	272·0	87·0	13·3	251·5	83·8	10·5	234·0
Cowpea	5 per cent. H_2O	86	542·5	91·4	19·7	398·0	88·0	4·5	362·5	84·5	4·0	333·5
Cowpea	5 per cent. M E	86	542·5	91·3	19·0	409·0	89·0	7·7	353·5	88·4	6·0	312·7
Sanai	5 per cent. H_2O	81	475·0	87·6	17·5	460·0	80·0	12·5	451·0	47·2	10·0	447·0
Sanai	5 per cent. M E	81	475·0	86·7	16·7	441·0	78·0	13·2	410·0	55·7	10·0	384·0
Ashoka leaves	5 per cent. H_2O	66	627·5	67·4	20·3	610·0	40·0	28·7	570·0	9·0	30·0	508·7
Ashoka leaves	5 per cent. M E	66	627·5	67·0	21·0	595·0	44·0	31·0	560·0	15·1	23·0	514·0
Dhaincha	5 per cent. H_2O	84	468·7	87·2	19·2	356·0	82·0	10·2	315·0	77·3	10·0	284·3
Dhaincha	5 per cent. M E	84	468·7	87·4	18·7	400·0	83·5	10·0	357·0	79·6	7·5	311·1

It will be noticed that the total nitrogen in the bulk of the green stuff steadily diminished during the period of the experiment. At the end of each month, at the time of sampling, portions of the fermented materials were added to soil in the proportion of 2 grms. per 100 grms. soil, and the several lots of soil incubated for three months, as in the earlier sets of experiments. The total nitrogen in soil was estimated after two months and again after three months.

The amount of nitrogen fixed was also calculated on the basis of the dry weight of the green stuff before fermentation. The results given in Tables IV, V and VI show that with maize, cowpea and *dhaincha*, the amount of nitrogen fixed after two months, per grm. of original material, on the dry basis, increased with the period of fermentation, but this was not true after three months' incubation. Continued fermentation, except of cowpea, depressed the amount of nitrogen fixed, expressed as the amount per gram of unfermented material, calculated on the dry basis. Fermentation of the green stuff for one month increased the nitrogen fixed when maize was added, to a high degree, and to a less extent when cowpea, *dhaincha* or *sanai* was added. When manure extract was added with the fresh material, the differences were less marked, except with cowpea, where the nitrogen fixed far exceeded that fixed on the addition of fermented cowpea.

TABLE IV.

Effect of one month's fermented green manures on nitrogen fixation in soil.

(*Mgms. of nitrogen per100 grms. of air-dry soil*)

Treatment	Nitrogen added as green manure 2 grms. net	Total nitrogen at start	After 2 months				After 3 months			
			Average total nitrogen in 100 grms.	Nitrogen fixed	Nitrogen fixed per 1 grm. of dry fermented green manure	Nitrogen fixed per 1 grm. of dry unfermented green manure	Average total nitrogen in 100 grms.	Nitrogen fixed in 100 grms. soil	Nitrogen fixed per 1 grm. of dry fermented green manure	Nitrogen fixed per 1 grm. of dry unfermented green manure
Maize (moisture 92 per cent.)	6·9	54·9	58·1	3·2	20·0	16·4	62·5	7·6	47·5	38·9
Maize (moisture 92·2 per cent.)	6·6	54·6	58·0	3·4	21·8	17·8	61·0	6·4	41·0	33·6
Cowpea (moisture 91·3 per cent.)	9·6	57·6	59·5	1·9	11·0	8·8	61·2	3·6	20·9	16·6
Cowpea (moisture 91·3 per cent.)	10·1	58·1	60·0	1·9	10·9	8·8	62·0	3·9	22·4	18·1
Sanai (moisture 86·7 per cent.)	11·1	59·1	61·0	1·9	7·7	6·3	63·5	4·4	17·7	14·6
Ashoka leaves (moisture 67·4 per cent.)	15·3	63·3	68·0	4·7	7·2	5·8	69·7	6·4	9·9	7·9
Ashoka leaves (moisture 67·0 per cent.)	15·0	63·0	69·0	6·0	9·1	7·1	70·0	7·0	10·6	8·4
Dhaincha (moisture 87·2 per cent.)	8·8	56·8	59·5	2·7	10·5	8·5	61·5	4·7	18·3	14·5
Dhaincha (moisture 87·4 per cent.)	9·8	57·8	61·0	3·2	12·7	10·3	63·0	5·2	20·6	16·7

TABLE V.

Effect of two month's fermented green manures on nitrogen fixation in soil.

(Mgms. of nitrogen per 100 grms. of air-dry soil)

Treatment	Nitrogen added as green manure	Total nitrogen at start	After 2 months				After 2 months			
			Average total nitrogen	Nitrogen fixed in 100 grms. soil	Nitrogen fixed per 1 grm. dry fermented green manure	Nitrogen fixed per 1 grm. dry unfermented green manure	Average total nitrogen	Nitrogen fixed in 100 grms. soil	Nitrogen fixed per 1 grm. dry fermented green manure	Nitrogen fixed per 1 grm. dry unfermented green manure
Maize . . .	7·55	55·5 0·5	62·0	5·6	32·5	23·5	63·5	8·0	40·0	28·8
Maize . . .	7·94	55·9 0·9	63·5	7·6	29·2	20·1	64·0	9·1	35·0	24·1
Cowpea . . .	9·57	57·5 0·6	61·0	3·4	14·1	10·6	63·0	5·4	22·5	17·1
Cowpea . . .	9·65	57·6 0·6	61·5	3·9	17·7	11·9	63·5	5·9	26·8	16·9
Sanai . . .	12·88	60·8 0·9	66·0	5·1	12·7	8·9	67·5	6·6	16·5	11·5
Sanai . . .	11·67	59·6 0·8	67·7	5·7	12·9	9·0	67·0	7·2	16·4	11·5
Ashoka leaves . .	22·05	76·0	76·0	6·0	5·0	2·5	78·0	8·0	6·7	3·4
Ashoka leaves . .	22·33	71·33 0·3	78·0	6·7	6·0	2·9	80·0	8·7	7·7	3·7
Dhaincha . . .	8·93	56·9 0·9	62·0	5·1	14·2	9·8	64·0	7·1	19·7	13·9
Dhaincha . . .	10·02	58·0 0·0	63·5	5·5	16·7	12·0	65·5	7·5	22·7	16·4

TABLE VI.

Effect of 3 months' fermented green manure on nitrogen fixation in soil, mgms. of nitrogen per 100 grms. of air-dry soil.

Treatment	Total nitrogen added as green manure	Total nitrogen at start	After 2 months				After 3 months			
			Average total nitrogen	Nitrogen fixed	Nitrogen fixed per 1 grm. dry fermented green manure	Nitrogen fixed per 1 grm. dry unfermented green manure	Average total nitrogen	Nitrogen fixed	Nitrogen fixed per 1 grm. dry fermented green manure	Nitrogen fixed per 1 grm. dry unfermented green manure
Maize . . .	8·3	56·3	64·0	7·7	30·8	19·4	65·0	8·7	34·8	21·9
Maize . . .	8·0	56·0	65·5	9·5	29·3	17·4	66·5	10·5	32·3	19·2
Cowpea . . .	9·4	57·4	64·0	6·6	21·3	15·0	64·5	7·1	22·9	16·2
Cowpea . . .	9·3	57·3	63·5	6·2	26·7	18·0	64·5	7·2	31·0	20·8
Sanai . . .	14·9	62·9	72·0	9·1	8·7	5·2	73·0	10·1	9·5	5·7
Sanai . . .	12·8	60·8	72·0	11·2	12·6	7·56	73·0	12·2	13·7	8·22
Ashoka leaves . .	57·3	105·3	104·5	—0·8	—0·44	—0·088	105·5	0·2	0·11	0·023
Ashoka leaves .	52·0	100·0	99·5	—0·5	—0·30	—0·075	100·5	0·5	—0·3	—0·075
Dhaincha . .	9·4	57·4	65·0	7·6	16·7	10·02	67·0	9·6	21·1	12·66
Dhaincha . . .	9·8	57·8	65·5	7·7	18·8	11·98	67·0	9·2	23·7	15·04

Experiments were next started to see whether pure cultures of nitrogen-fixing organisms could utilise as a source of energy the products formed when plant tissues were acted on by cellulose-dissolving organisms.

Three kinds of plants were used, namely cowpea, maize, and *ashoka* leaves. The materials were dried, powdered, and one gram weight put into flasks containing 100 c. c. of the medium containing.

$(NH_4)_2SO_4$	2·0 per cent.
K_2HPO_4	0·5 per cent.
$MgSO_4$	0·2 per cent.
NaCl .. .. —	0·1 per cent.
$CaSO_4$	0·1 per cent.
Soil (calcareous)	1·0 per cent.

After introduction of the plant tissue the flasks were sterilized, inoculated with pure culture of cellulose-dissolving organisms incubated for one month at 30° C. Duplicate flasks were then inoculated with pure cultures of nitrogen-fixing organisms, incubated for 3 weeks, and the total nitrgeon estimated. The results are shown in Table VII.

TABLE VII.

Nitrogen fixation in solution with green manure.

Organisms	Cowpea		Maize		*Ashoka*	
	Total nitrogen	Nitrogen fixed	Total nitrogen	Nitrogen fixed	Total nitrogen	Nitrogen fixed
Control	24·7, 24·3 } 24·5	..	19·20, 18·10 } 18·65	..	30·25, 30·65 } 30·45	..
Azotobacter	25·2, 25·8 } 25·5	1·00	19·7, 19·6 } 19·65	1·00	29·60, 30·70 } 30·15	—0·30
Nodule organism	26·4, 26·6 } 26·50	2·60	19·7, 19·9 } 19·80	1·15	31·00, 31·25 } 31·125	0·675
Manure organism	25·85, 25·90 } 25·875	1·375	18·5, 19·5 } 19·00	0·35	30·90, 31·00 } 30·95	0·50
Clostridium	26·3, 26·3 } 26·3	1·80	19·8, 20·7 } 20·25	1·60	31·40, 31·25 } 31·325	0·875

A further experiment was carried out with sann hemp that had been rotten in a pit for three months.

To 100 c. c. of a medium, similar to the one used in the previous experiment except that 0·5 grm. calcium carbonate was substituted for soil, were added 2 grms.

of farm yard manure : a set of five flasks was taken two of which were sterilised as controls, and the other three incubated for 4 weeks, after which nitrogen was estimated in all the flasks. To a second set of five flasks were added 2 grms. farmyard manure and 1 grm. rotted sann hemp. Of this set also, two flasks were sterilised and kept as controls, and the other three incubated for 4 weeks, and nitrogen estimated. The results for the two sets are given in Table VIII.

TABLE VIII.

Nitrogen fixation with rotted green manure in solution.

	Basal medium and 2 grms. farmyard manure	Average	Basal medium and 2 grms. farmyard manure and 1 grm. rotted sann hemp	Average
		Mgms.		Mgms.
Control	13·9 14·0	13·95	34·2 34·1	34·15
Incubated flasks . . .	14·2 14·3 14·4	14·3	35·5 35·6 35·5	35·53
Nitrogen fixed . . .	..	0·35		1·40

The influence of green, immature plant residues on nitrogen fixation having been tested, in the next series straw, fresh and fermented, was used.

The straw was fermented by adding 2 per cent. ammonium carbonate and keeping moist for three months, by which time the weight had diminished to about one half of the original.

Before mixing with the soil, the fermented straw was dried and pulverised. It was added at the rates of one per cent. and 2 per cent. to duplicate lots of soil.

The fresh straw, dried and powdered, was supplied at the rate of 2 per cent. to duplicate lots of soil.

The nitrogen content of the fresh straw was 6·5 mgms. per gram and of the fermented 13·4 mgms.

The soil was brought to optimum moisture content, loss of moisture made up every week, and nitrogen estimated at the end of two, three and four months.

The results given in Table IX show that maximum fixation of nitrogen was obtained by the addition of one per cent. fermented straw.

TABLE IX.

Effect of fermented and unfermented straw on nitrogen fixation in soil, mgms. of nitrogen per 100 grms. of air-dry soil. Original nitrogen in soil 42·5.

Number of jars	Treatment	* Total nitrogen at start	After 2 months			After 3 months			After 4 months		
			Average total nitrogen	Nitrogen fixed	Average nitogen fixed	Average total nitrogen	Nitrogen fixed	Average nitrogen fixed	Average total nitrogen	Nitrogen fixed	Average total nitrogen
Jar 1	2 per cent. fermented straw	69·3	84·0	14·7	14·1	89·2	19·9	19·3	87·5	18·2	18·7
Jar 2	2 per cent. fermented straw	69·3	82·7	13·4		88·0	18·7		88·5	19·2	
Jar 3	1 per cent. fermented straw	55·9	70·2	14·3	16·2	70·5	14·6	16·6	77·0	21·1	20·3
Jar 4	1 per cent. fermented straw	55·9	74·0	18·1		74·5	18·6		75·0	19·5	
Jar 5	2 per cent. unfermented straw	55·5	65·0	10·0	6·0	66·5	11·0	9·2	69·5	14·0	13·0
Jar 6	2 per cent. unfermented straw	55·5	57·0	2·0		63·0	7·5		67·5	12·0	

* Total nitrogen of soil and nitrogen of manures added.

At the same time as the nitrogen-fixation experiment, pot-culture experiments were carried on, in which one per cent. and 2 per cent. fermented, and 2 per cent. fresh straw was added to the soil and oats grown. As is usually found the addition of straw seriously depressed the yield. The addition of fermented straw increased the yield by one-sixth; there were no significant differences between the yields when one per cent. or 2 per cent. was added.

TABLE X.

The results of the pot experiment with fermented and unfermented straw (oats).

Pot number	Treatment	Weight of whole plants grms.	Weight of straw grms.	Weight of grain grms.	Number of tillers fertilised	Total number of tillers
1	Control	29·0	16·0	11·5	8	9
2		28·0	16·0	11·0	8	11
3		27·0	14·0	10·5	6	7
Average		28·0	15·33	11·0	7	9
4	Unfermented straw 2 per cent.	16·0	6·5	4·5	6	7
5		11·5	5·0	2·5	4	4
6		11·0	5·0	2·0	4	9
Average		12·83	5·5	3·0	5	6
7	Fermented straw one per cent.	30·5	16·5	13·0	10	13
8		29·0	16·0	12·0	10	14
9		32·5	16·5	13·5	11	14
Average		31·66	16·33	12·83	10	13
10	Fermented straw 2 per cent.	31·5	15·0	13·0	10	15
11		30·0	13·5	12·0	10	12
12		37·0	18·5	14·5	8	8
Average		32·83	15·66	13·16	9	11

Two series of experiments were also carried out to test the utility of fermented straw as a source of energy for nitrogen-fixing bacteria in pure culture.

A basal medium was made up consisting of—

K_2HPO_4	0·2 grm.
$MgSO_4$	0·04 grm.
$(NH_4)_2SO_4$	0·1 grm.
$CaCO_3$	10·0 grms.
$CaSO_4$	0·002 grm.
Water	1,000 c. c.

Fifty c. c. of the medium were distributed into each of ten flasks, and 1 grm. well rotted farmyard manure and 5 grms. straw added to each flask, and incubated at 30° C. for two months.

The flasks were then sterilised, a pair kept as controls, and other pairs inoculated with azotobacter, a nodule organism, *clostridium*, and a nitrogen-fixing organism from manure respectively.

Duplicate flasks of Ashby's mannite solution were also inoculated with these organisms (Table XI). For comparison concurrently with the first series of flasks, another series was set up, in which the basal medium was composed of—

K_2HPO_4	1·0 grm.
$MgSO_4$	0·2 „
NaCl	0·2 „
$CaSO_4$	0·5 „
Water	1,000 c.c.

Ten flasks each containing 100 c.c. of this medium, were taken and 0·5 grm. calcium carbonate 0·5 grm. fermented manure and 1·0 grm. powdered straw added to each. After sterilisation the flasks were inoculated with cellulose-destroying organisms and incubated for one month, and then pairs of flasks inoculated as in the

other series. The results are shown in Table XI.

TABLE XI.

Effect of straw on nitrogen fixation in different solutions.

Nitrogen fixer used	Fermented manure 1 grm. Powdered straw 5·0 grms.			Pure culture cellulose organisms. Fermented manure 0·5 grm. Powdered straw 1 grm.			
	Total nitrogen	Nitrogen fixed	Nitrogen fixed per grm. of mannite in Ashby's solution	Total nitrogen	Average total nitrogen	Nitrogen fixed	Nitrogen fixed per grm. of mannite in Ashby's solution
	Mgms.	Mgms.		Mgms.		Mgms.	
Control	55·8, 56·6 — 55·7	..	Run concurrently	12·0, 11·6	11·8	..	..
Azotobacter	60·8, 61·6 — 61·2	5·5	2·975	13·6, 13·5	13·55	1·75	2·975
Nodule organisms	56·9, 56·9 — 56·9	1·2	0·975	12·9, 12·5	12·7	0·90	0·975
Manure organisms	60·15, 60·80 — 60·4	4·7	1·275	13·5, 13·5	13·5	1·7	1·275
Clostridium	66·7, 66·6 — 66·65	10·95	0·875	11·80, 12·5	12·15	0·35	0·875

The nitrogen fixation with 5 grms. fermented straw is higher than Ashby's solution for each organism, especially for the *clostridium*. The medium containing one gram of straw fermented by the pure culture of cellulose destroyers was superior to Ashby's, only with the nitrogen-fixing organism that had been isolated from manure.

This organism had been isolated from cow and horse dung, which had been found to fix nitrogen in a series of experiments described below :—

Five grams of the dung with 0·5 grm. calcium carbonate and 100 c.c. water, were placed in duplicate flasks, and another pair of flasks was taken in which Ashby's mannite solution containing one grm. mannite replaced the water. Each flask was connected to an ammonia trap, containing 200 c.c. of $N/14$ sulphuric acid and air drawn through flasks so as to bubble slowly through the acid.

The nitrogen in the traps and flasks was estimated at the end of 21 days, and the results are shown in Table XII.

TABLE XII.

Nitrogen fixation in cow and horse dung by continuous aeration for 21 days.

Treatment	Original nitrogen Mgms.		NH_3 in traps Mgms.	Nitrogen in flasks Mgms.		Total nitrogen Mgms.	Nitrogen fixed Mgms.		Nitrogen fixed per grm. manure Mgms.	Average per cent.	Difference between cow and horse dung	Difference between tap water and Ashby's solution
Cow dung — Tap water	33·0	33·0	17·4	25·5	25·8	33·2	·2	·5	0·1	1·68	Tap water	Cow dung
	33·0			26·1								
			29·1	24·5	24·8	33·9	·9					
				25·1								
Cow dung — Ashby's solution	33·0	33·0	17·5	30·0	29·9	37·4	4·4	4·4	0·9	13·2	Tap water 20·68 per cent. more for horse manure	
				29·8								
	33·0		29·1	28·5	28·3	37·4	4·4					
				28·1								
Horse dung — Tap water	38·0	38·0	13·6	42·5	42·6	46·2	8·2	9·5	1·9	22·36	Ashby's solution 19·5 per cent. more for horse manure	Horse dung
				42·7								
	38·0		25·8	43·0	43·0	48·8	10·8					
				43·0								
Horse dung — Ashby's solution	38·0	38·0	16·3	44·0	44·0	50·3	12·3	12·1	2·4	32·7		10·34
				44·0								
	38·0		2·68	43·0	43·0	49·8	11·8					
				43·0								

Appreciable quantities of nitrogen were fixed by horse dung both in tap water and in Ashby's solution. The amount of nitrogen fixed by cow dung in tap water was slight but that in Ashby's solution was eight times as much as in tap water. The increase in the amounts of nitrogen fixed due to addition of Ashby's mannite solution was the same either with horse dung or cow dung. From both lots of cow dung the quantity of ammonia evolved amounted to about 25 per cent. of the total nitrogen, while that from horse dung was not so great. The horse dung gains in total nitrogen when properly aerated even when the ammonia lost is not taken into account.

A mixture of horse and cow dung manure alone and with addition of straw was then tested for nitrogen fixation under aerobic conditions. One hundred grms. of manures were placed in each of the duplicate flasks, provided with ammonia traps and air drawn through as in the previous experiment. The nitrogen was estimated after 3 weeks' continuous aeration and the results are shown in Table XIII.

TABLE XIII.

Fixation of nitrogen in cattle manure and manure littered with straw by continuous aeration for 21 days.

Treatment	No.	Amount of manure taken	Amount of straw	Total amount	Total nitrogen in each flask at start	After 21 days				
						Ammonia in traps	Nitrogen in flasks	Total nitrogen	Fixation of nitrogen in each flask	Fixation of nitrogen per 100 grms. manure
		Grms.	Grms.	Grms.	Mgms.	Mgms.	Mgms.	Mgms.		
Manure	1	100	*Nil*	100	330·0	3·3	340·2	345·5	13·5	23·0
	2	100	*Nil*	100	330·0	3·1	359·3	362·4	32·4	
Manure straw	3	30	10	40	184·0	2·0	192·1	194·1	10·1	30·0
	4	30	10	40	184·0	1·9	189·9	191·8	7·8	

Distinct gains of nitrogen were again found and show significant effect of the addition of straw, nitrogen evolved as ammonia is much smaller than that fixed in the flasks. The added straw appears to supply the energy and to provide better aeration for nitrogen-fixing organisms.

CONCLUSIONS.

The experiments detailed in the foregoing pages definitely show that the fixation of nitrogen does take place when green manures, farmyard manure, straw or any other organic material is added to the soil under favourable conditions of temperature and moisture.

In almost all field experiments where the nitrogen balance-sheet is drawn after removal of the crops, the balance is favourable and indicates a gain in soil nitrogen, allowance being made for the nitrogen removed by the crops. The experiments detailed here can explain how this gain of nitrogen is brought about. Almost all forms of organic material help the nitrogen-fixing organisms of the soil and if sufficient time is allowed for their activity, the nitrogen content of the soil is increased. The crops grown immediately after the application of fermented green or dry organic manure derive much nutrition since the nitrogen of such manures is easily available. Where unfermented organic manures are used, time has to be allowed for their decomposition in the soil before the crop following such application can derive any benefit. The enrichment of soil in nitrogen brought about by unfermented manures is not equalled by fermented manures, nitrogen fixation with unfermented manures being generally higher than with fermented manures, provided sufficient time is allowed for the decomposition of the former in the soil. The tissues of non-leguminous plants ultimately add much more atmospheric nitrogen than those of leguminous plants, when fermentation with soil is accelerated by the addition of manure extract. The total nitrogen of the soil is brought up to the same level in both the treatments because although the leguminous plants are much richer in nitrogen at the time of addition to the soil, the subsequent nitrogen fixation in the case of non-leguminous plants is much greater.

The fermented tissues of non-leguminous plants are much more beneficial than those of leguminous plants for fixing atmospheric nitrogen. Artificial farmyard manure prepared by fermenting straw was found to fix in the soil a fairly large amount of nitrogen from the air and to benefit the plants to a great extent as seen by the pot-culture experiments.

Various experiments conducted in artificial media show the different organisms that are responsible for the addition of atmospheric nitrogen and the materials which serve as energy for the nitrogen fixation by them. Materials like cow and horse dung which are known to lose nitrogen on keeping were found to fix atmospheric nitrogen when they were aerated and loss of nitrogen as ammonia recovered from them and taken into account. Aeration of soil proved to be the main factor responsible for nitrogen fixation. This shows that the proper cultivation of the soil which brings about aeration, and thereby addition of nitrogen, is of great importance for maintaining the level of soil nitrogen and for the ultimate benefit of the crops.

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THE CLINGING POWER OF COTTON AND THE NUMBER OF CONVOLUTIONS PER CENTIMETRE.

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(With six text-figs.)

I. Introduction.

In the course of examination of cotton obtained from each week's picking, it was found that there was a large variation in the number of convolutions per centimetre. As Adderley [1922] had found "that the clinging power of cotton hairs depends upon the convolutions", it was thought that a study of the former property in these samples would be of interest. But it has been shown by Navkal and Turner [1930] that the clinging power by itself has a negative correlation with the spinning value, while the property called the clinging power per whole fibre per unit fibre-weight per inch has a positive correlation with it. In order, therefore, to understand the effect on the spinning value, it is the latter property that should be taken into consideration. As in the calculation of this property the fibre-length and the fibre-weight are to be known, these values were also determined in addition to the clinging power. The present paper deals with this study.

II. Material and sampling.

During February and March of 1930, samples were collected from the weekly pickings of Cambodia Co. 2 (*G. hirsutum*) grown in the unmanured plots of field No. 5a of the Cotton Breeding Station, Coimbatore. With a view to reduce the variation within the samples to a minimum, care was taken to select only nine-seeded locks from four-locked bolls bursting in each week. Even among the seeds in the lock a further selection of the fourth and fifth positions was made to get a uniform sample. The eight samples thus obtained formed the material for the present enquiry.

III. Experimental methods.

The fibre-length was determined by means of the Balls' Sledge Sorter. Generally two tests* were made, but if sufficient agreement was not observed between the two, one or two more determinations were made and the mean of all was taken. The fibre-weight was determined by cutting centimetre lengths and weighing them in a quartz-fibre-torsion-micro-balance as described in the Technological Reports

* A separate sliver was prepared for each test.

on Standard Indian Cottons [Turner, 1928]. The determination of the convolutions was also carrried out as in the above-mentioned reference, by counting the total number of convolutions over the entire length of the fibre and dividing it by the length. Fifty fibres were examined for each sample. Though a much larger number is required according to Koshal and Turner [1930], this number was deemed sufficient in the present case, because the variability in these samples was very small and the differences were found to be significant (Table I). It may be mentioned that the small variation observed in the present samples should be due to the elimination during sampling of many of the factors that go to widen the variation.

The method followed in the determination of the clinging power was exactly the same as that described by Navkal and Turner [1930], except for the fact that instead of using calcium chloride solutions giving appropriate relative humidities in the O'Neill tube, water was employed. This modification had to be made in spite of its drawbacks, because the use of the solution was found to soil the pads badly, especially when the same pair of pads had to be used for testing all the eight samples, as will be described below.

The clinging power determined in the present work is of two kinds. One is the normal clinging power which is obtained when the fibres in the pads and those that are slipped through them are derived from the same sample. The other is the clinging power determined by keeping a single pair of pads constant and slipping through them the fibres from all the other samples. The object in making the latter measuremenvs is to study the fluctuation that would be caused in the value of the clinging power, when there is difference in the average number of convolutions between the fibres in the pads and those that are slipped.

IV. Discussion of results.

Fibre-length, fibre-weight and convolutions.—The values of these properties are given in Table I.

Table I.

No. of picking	Fibre-length in inches	Fibre-weight per cm. 10-6 grm.	No. of convolutions per cm.	S. D. of convolutions
1	1·03	1·92	58	1·95
2	1·04	1·87	60	1·86
3	1·03	1·87	47	1·84
4	1·03	1·88	39	1·25
5	1·03	1·81	35	1·48
6	1·01	1·77	30	1·24
7	0·95	1·85	22	1·12
8	0·94	2·04	20	0·98

The fibre-length is practically constant except in the last two pickings where it shows a sudden fall. The fibre-weight also does not appear to show much fluctuation. On the other hand the number of convolutions varies a good deal, steadily decreasing as the picking advances. All the differences except two, *viz.*, those between the first and second and the seventh and eight pickings are significant. The difference between the maximum and the minimum is as high as two-hundred per cent. of the latter or sixty-six per cent. of the former.

Clinging power.—In discussing this property the following procedure is adopted. The values of the clinging power when a single pair of pads is used for testing all the samples are taken up first. After examining the three sets of results one after the other and summing up all the three, the case of identical sample both for the pads and slipping fibres is considered.

Convolutions in pads, 39.—This case corresponds to a certain extent with that of Adderley's [1922], for fibres having a varying average number of convolutions, varying as they do from 20 to 60,* are pulled against pads which contain on the average 39 convolutions, that is, about the mean of the range under study.

The results obtained are given in Table II.

TABLE II.

Convolutions in pads, 39.

No. of picking	Convolutions per cm.	Fibre-weight per cm. 10^{-6} grm.	Clinging power grms.		Clinging power per unit surface	C. pr. × length (Fibre-weight per cm.)
			Value	Standard error		
1	60	1·87	5·30	0·13	3·38	7·86
2	58	1·92	5·32	0·15	3·84	7·61
3	47	1·87	5·53	0·14	4·04	8·12
4	39	1·88	5·98	0·15	4·36	8·74
5	35	1·81	5·65	0·13	4·20	8·57
6	30	1·77	5·55	0·13	4·17	8·45
7	22	1·85	5·30	0·13	3·90	7·26
8	20	2·04	5·63	0·13	3·94	6·92

* The range of half convolutions in Adderley's [1922] work was between 20 and 90.

It will be seen that the clinging power increases as the convolutions increase and attains a maximum when the slipping fibres possess the same number of convolutions as those in the pads and then decreases though the convolutions increase. It is no doubt true that all the differences are not statistically significant but nevertheless there can be no denial of the shape of the curve (Fig. 2), *viz.*, the rise and the fall.

It is interesting to note that these results are in conformity with those of Adderley [1922] who also obtains curves similar to the present.

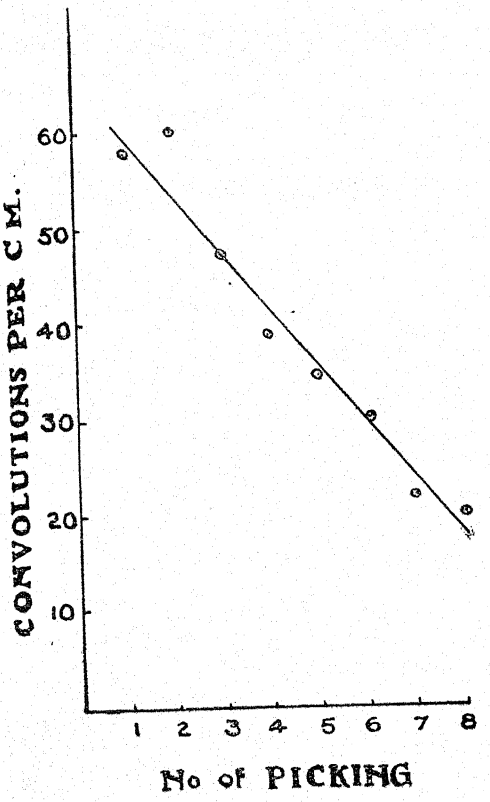

Fig. 1.

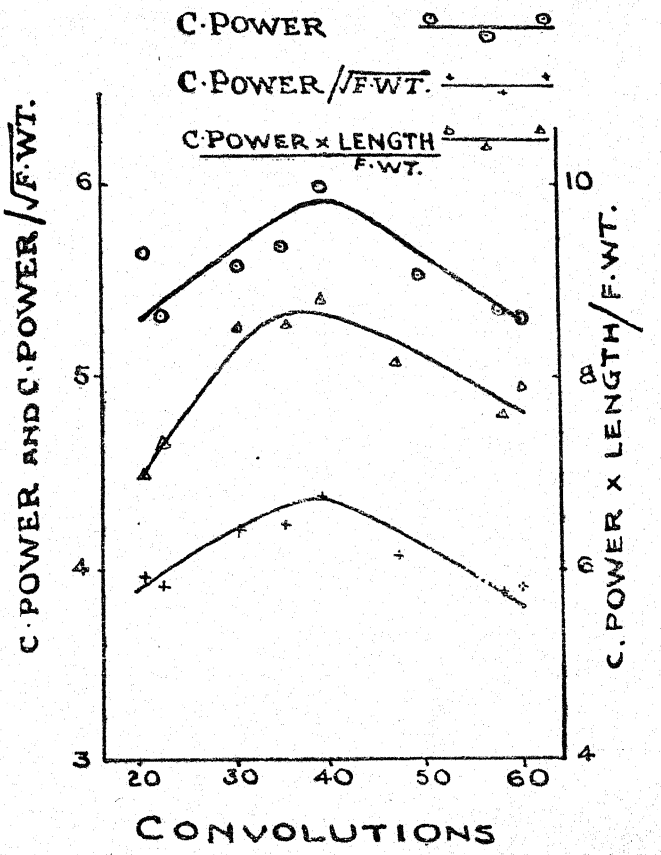

Fig. 2.

It will be seen in Fig. 2 that the value of the clinging power for convolutions 20 is significantly higher than the corresponding value on the smoothened curve. This may be due to the high value of the fibre-weight obtained for that sample. The error due to the variation of this factor may, as pointed out by Navkal and Turner [1930], be eliminated by dividing the value for the clinging power by the square root of the corresponding fibre-weight, when an approximate measure of the clinging power per unit surface area is obtained. The points representing this property also lie on a curve similar to the above but they show less divergence from the smoothed out curve than do those of the bare clinging power (Fig. 2).

The values of the clinging power obtained above may be used to examine what the effect of mixing cottons with different number of convolutions will be on the spinning value. It was found by Navkal and Turner [1930] that the clinging power had a negative correlation with the spinning value, while the correlation co-efficient between the latter and the clinging power per unit surface area was only—0·1 which is insignificant. On the other hand, it was found that the correlation co-efficient between the quantity called the clinging power per whole fibre per unit fibre-weight per inch and the spinning value was as high as +0·6, which is fairly significant. It is clear, therefore, that in the determination of the spinning performance of a cotton, it is this quantity that should be taken into consideration. The last column of Table II and the central curve of Fig. 2 show these values. It will be seen that these values also lie on a curve similar to the other two but on account of the increased variability many of the differences are not significant. It is fairly clear, however, that there is a tendency for the occurrence of higher spinning values when cottons having nearly the same number of convolutions are mixed.

Convolutions in pads, 60.—Having found that the form of the curve of clinging power is a rise and a fall when the pads contained fibres having about the mean number of convolutions of the range, we may now consider what it will be when the extreme samples are used for the pads. The case when this sample has

the maximum convolutions of the range will be considered at present. The results are given in Table III and represented graphically in Fig. 3.

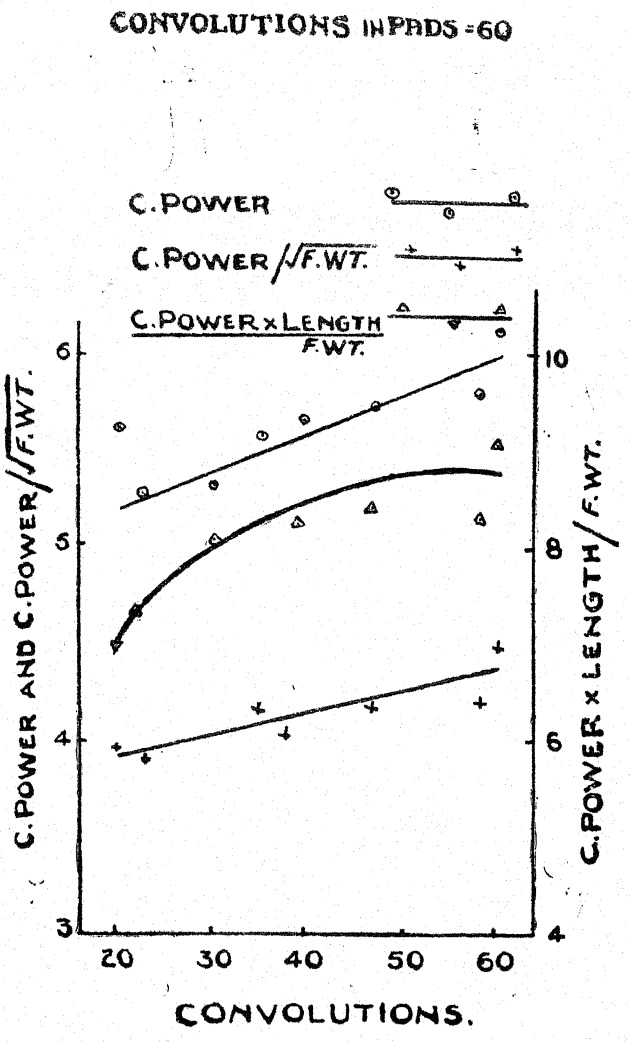

Fig. 3.

TABLE III.

Convolutions in pads, 60.

No. of picking	Convolutions per cm.	Fibre-weight per cm. 10^{-6} grm.	Clinging power grms.		Clinging power per unit surface	C. pr. × length / (Fibre-weight per cm.)
			Value	Standard error		
2	60	1·87	6·16	0·16	4·50	9·14
1	58	1·92	5·81	0·16	4·19	8·31
3	47	1·87	5·74	0·13	4·19	8·43
4	39	1·88	5·67	0·15	4·14	8·28
5	35	1·81	5·59	0·16	4·15	8·48
6	30	1·77	5·32	0·15	4·00	8·10
7	22	1·85	5·30	0·14	3·90	7·26
8	20	2·04	5·63	0·14	3·94	6·92

The clinging power is a maximum when the fibres tested possess the same number of convolutions as the pads do, and decreases as the convolutions decrease. The form of the curve is nearly a straight line. Here also, as in the previous case, all the differences are not reliable but there can be no denial of the fall.

Dividing the clinging power by the square-root of the fibre-weight does, as before, lessen the divergence from the smoothed out curve.

As regards the quantity, clinging power per whole fibre per unit fibre-weight per centimetre, all the values obtained are nearly the same except those for the last two pickings which are significantly smaller. This means that mixing cottons widely differing in the convolutions may tend to reduce the spinning value, while mixing of the others produces very little effect.

Convolutions in pads, 22.—The case when the number of convolutions in the pads is about the minimum of the range may now be considered. Table IV and Fig. 4 represent the results.

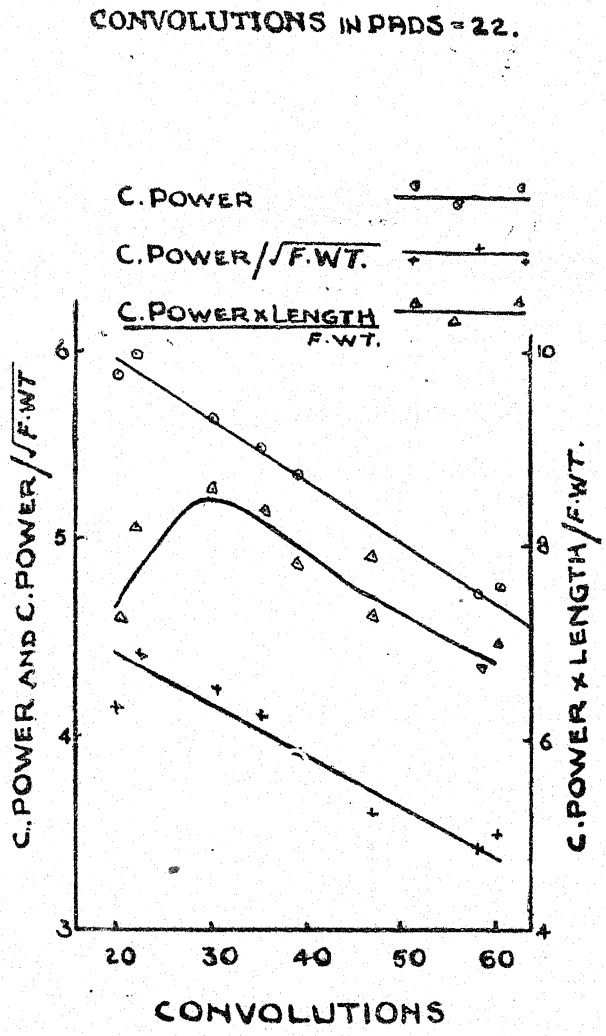

Fig. 4.

TABLE IV.

Convolutions in pads, 22.

No. of picking	Convolutions per cm.	Fibre-weight per cm. 10^{-6} grm.	Clinging power in grms.		Clinging power per unit surface grms.	C. pr. × length / (Fibre-weight per cm.)
			Value	Standard error		
2	60	1·87	4·79	0·11	3·50	7·10
1	58	1·92	4·74	0·14	3·42	6·78
3	47	1·87	4·94	0·14	3·61	7·26
4	39	1·88	5·36	0·13	3·91	7·83
5	35	1·81	5·50	0·13	4·09	8·35
6	30	1·77	5·64	0·14	4·24	8·58
7	22	1·85	5·98	0·13	4·39	8·19
8	20	2·04	5·88	0·13	4·12	7·23

It will be seen that here also the clinging power is a maximum when the fibres in pads and those that are tested are derived from identical sample. It decreases as the differences between the number of convolutions in the two increases. The curve is a straight line similar to the previous one but with a slope in the negative direction.

The figures for the clinging power per whole fibre per unit fibre-weight per cm. show that the maximum instead of corresponding to 22 convolutions does to 30. Moreover, the value for the case of identical pad and sample tested does not differ considerably from that for the others. It follows, therefore, that the spinning value is not considerably affected by any of the different mixings.

Same pad for all samples.—The three cases considered above possess a number of common features which may be summed up as follows.

The clinging power is uniformly maximum when the fibres in the pads and those tested are derived from identical sample, a fact which holds true whatever may be the number of convolutions for the sample.

When the fibres tested do not possess the same number of convolutions as the pads do, there is a fall in the value of the clinging power. This fall is always positive whatever may be the sign of the difference in convolutions.

The fall increases as the difference increases. This can be clearly seen in Table V which gives the differences in the clinging power as well as the clinging

power per unit surface area corresponding to those in the number of convolutions. The same is shown graphically in Fig. 5.

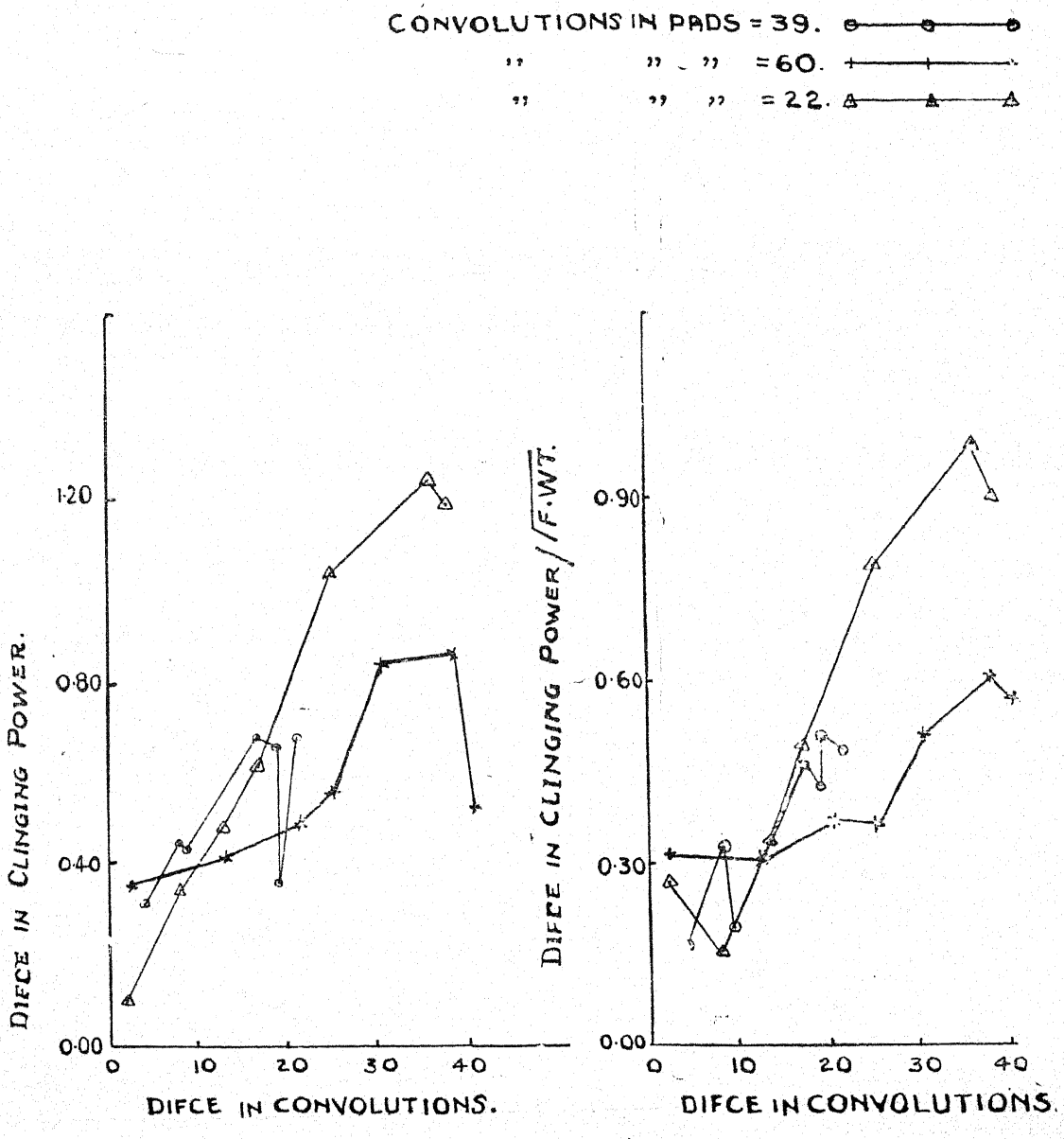

Fig. 5

TABLE V.

Differences in clinging power corresponding to those in the number of convolutions.

Pad convolutions, 39			Pad convolutions, 60			Pad convolutions, 22		
Convolutions	Clinging power	Clinging power per unit surface	Convolutions	Clinging power	Clinging power per unit surface	Convolutions	Clinging power	Clinging power per unit surface
—4	0·33	0·16	—2	0·35	0·31	—2	0·10	0·27
8	0·45	0·32	—13	0·42	0·31	8	0·34	0·15
—9	0·43	0·19	—21	0·49	0·37	13	0·48	0·32
—17	0·68	0·46	—25	0·57	0·35	17	0·62	0·49
—19	0·35*	0·42	—30	0·84	0·50	25	1·04	0·79
19	0·66	0·52	—38	0·86	0·60	36	1·24	0·98
21	0·68	0·49	—40	0·53*	0·56	38	1·19	0·90

When medium twisted yarns were considered, it was found by Navkal and Turner [1930] that the yarn strength and the clinging power were negatively correlated. But in soft twisted yarns, where the effect of twist is not marked, it may be possible that the strength of the yarn depends on the clinging power. In such a case it follows from the above results that the selection for mixing of cottons possessing nearly the same number of convolutions would tend to increase the strength of such a yarn.

A tendency towards a similar effect of such a mixing on the spinning value, as determined by the highest standard warp count, is also noticeable but the conclusion is not always true.

* These values show some discrepancy, which may be accounted for by the high value of fibre-weight obtained for these cases. On elimination of this factor the discrepancy is found to vanish as can be seen in the corresponding figures in the next column.

Pads and fibres tested from identical samples.—The variations considered above do not represent those that occur in common practice because in those cases fibres having varying number of convolutions were tested with pads having some arbitrary average number. In order to correspond to a normal case, fibres in the pads as well as those that are pulled must be derived from identical samples. It would then give the effect of the convolutions as would occur in common practice. Such a case will be considered at present. Fig. 6 and Table VI represent the results for the eight samples when the pads and fibres examined were obtained from identical samples.

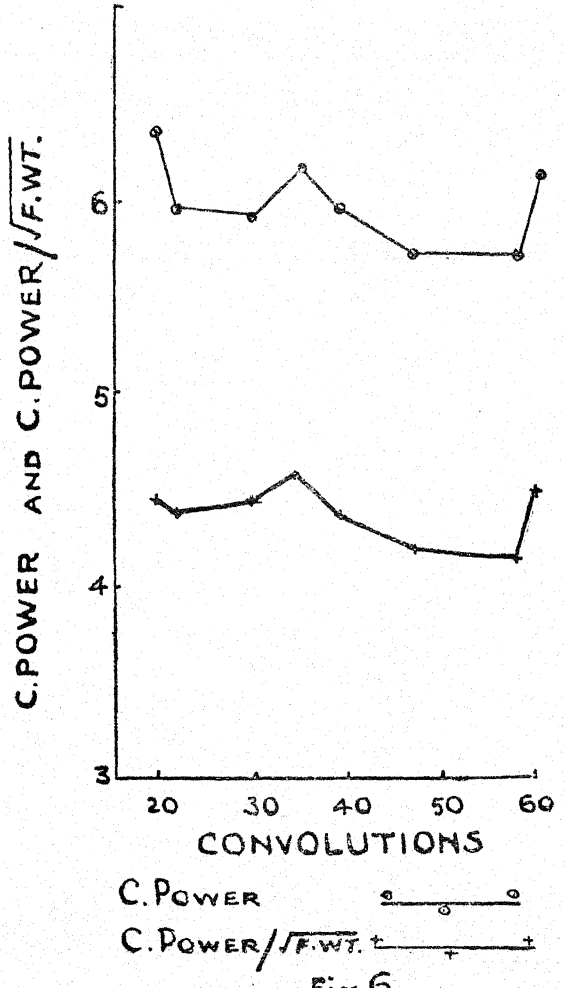

Fig. 6.

TABLE VI.

Pads and fibres from identical samples.

No. of picking	Convolutions per cm.	Fibre-weight per cm. 10^{-6} grm.	Clinging power in grms.		Clinging power per unit surface
			Value	Standard error	
1	58	1·92	5·76	0·20	4·14
2	60	1·87	6·16	0·16	4·50
3	47	1·87	5·73	0·12	4·19
4	39	1·88	5·98	0·15	4·36
5	35	1·81	6·16	0·18	4·58
6	30	1·77	5·92	0·15	4·45
7	22	1·85	5·98	0·13	4·39
8	20	2·04	6·34	0·16	4·43

Though the individual values of the clinging power in this case are of nearly the same order as was found to exist in the other three cases, it is interesting to find that no systematic and orderly variation, as was observed therein, is perceptible here. Except for the last value, which is significantly larger, all the others are nearly the same. Even this exception vanishes when correction is applied for the variation in the fibre-weight, thus showing that it is due to the abnormal value of the latter factor. It follows therefore that when the pads and fibres are from identical sample, the clinging power (other properties being equal) remains the same whatever may be the number of convolutions. In other words, convolution has no correlation with the normal clinging power. The value of the correlation co-efficient between these properties is $-0{\cdot}38 \pm 0{\cdot}20$. As the co-efficient is only a little greater than its error no importance can be attached to it. There is no improvement either when the variation in fibre-weight is corrected, for even then the co-efficient is only $-0{\cdot}34 \pm 0{\cdot}21$.

The result, *viz.*, that the variation in the convolutions has no effect on the normal clinging power, may probably account for the fact that no correlation has been observed so far between the convolutions and the yarn strength.

V. CONCLUSIONS.

(1) When the same pair of pads is used for testing all the samples,

(*a*) the clinging power is a maximum when the fibres tested and those that form the pads are derived from the same sample,

(*b*) if the convolutions in the two are different there is always a fall in the clinging power; this fall increases with increasing difference irrespective of the sign,

(*c*) the elimination of the variation in fibre-weight will make the results agree better with the conclusions enunciated above.

(2) A possible consequence of the above-mentioned conclusions may be that the strength of soft twisted yarns tends to increase, if cottons possessing nearly the same number of convolutions are selected for mixing.

A similar effect of such a mixing on the spinning value, as determined by the highest standard warp count, is also noticeable but the conclusion is not always true.

(3) When the fibres in the pads and those tested are derived from identical samples, the clinging power bears no relationship with the convolutions, which probably accounts for the fact, that no correlation has so far been obtained between the yarn-strength and the convolutions.

VI. Acknowledgments.

The writer wishes to express his thanks to Mr. V. Ramanatha Ayyar, Cotton Specialist, Coimbatore, for permission to carry out this work as well as for his very valuable suggestions. His special thanks are due to Dr. Nazir Ahmad, Director, Cotton Technological Research Laboratory, Matunga, for the supply of apparatus, for reading through the manuscript and for the very valuable improvements he suggested in the preparation of the paper.

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A NOTE ON A CROSS OF *GOSSYPIUM STOCKSII* M. MAST WITH *GOSSYPIUM INDICUM* GAMMIE.

BY

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(With Plates XXII and XXIII)

Introduction.

This note describes the parents and F_1 generation from an interspecific *Gossypium* cross, which does not appear to have been described before, though Leake and Prasad [1911] have reported the results of closely related crosses.

The F_1 generation in the cross here described showed considerable differences between the different plants, and it seems this fact is of interest and worthy of record, even if it has not been possible to explain it. The complete sterility of the F_1 generation has prevented any further generations being studied.

It would be desirable to undertake a cytological examination of the material, but this has not so far been possible.

The possibilities of back-crossing on to the parent *indicum* plant were not explored. That this might be a promising line of enquiry is shown by a recent note by Harland [1932].

It should be mentioned that the breeding in of the two parents gives no suggestion of 'impurity' on either side.

Material.

PARENTS OF THE CROSS.

G. stocksii *M. Mast* (*Plate XXII, fig. 1*).

The seed of this cotton was obtained from Mr. K. I. Thadani in Sind. Considerable difficulty was experienced in getting the seeds to germinate. It was, however, found* that immersion of seeds in concentrated sulphuric acid for 24 hours and subsequent soaking in water for 24 hours made the testa sufficiently soft to allow it to be peeled off. The embryo is then placed on filter paper soaked

* This method was successfully tried by Mr. Akbar Ali Tur, Technical Assistant, Cotton Research Laboratory, Lyallpur.

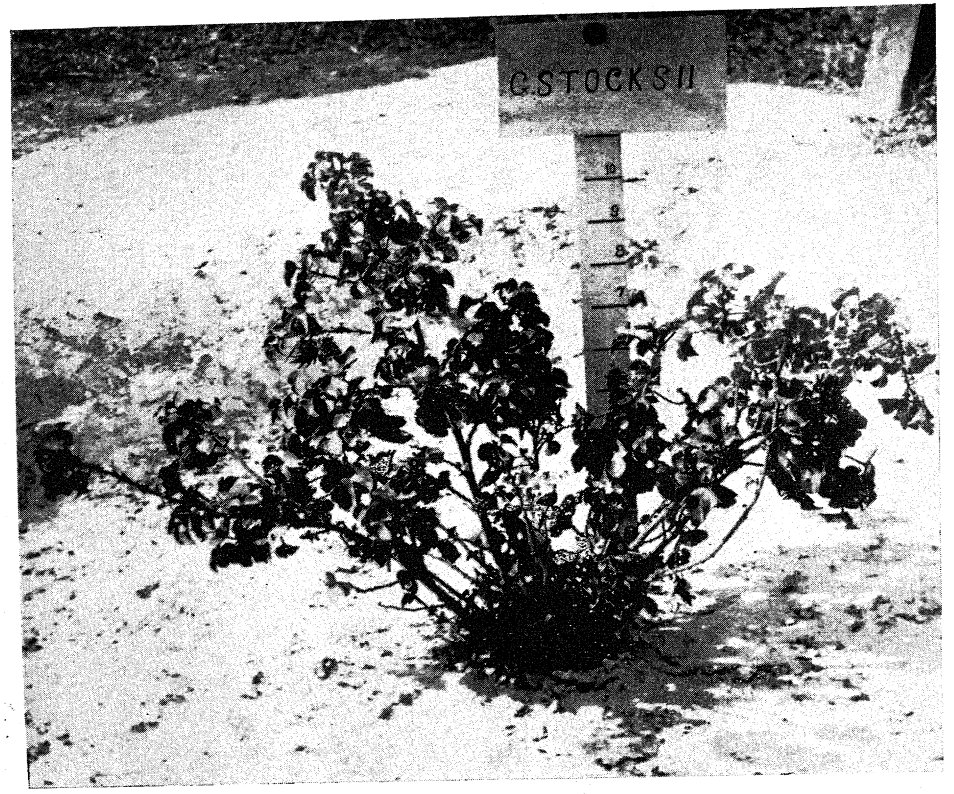


Fig. 1.

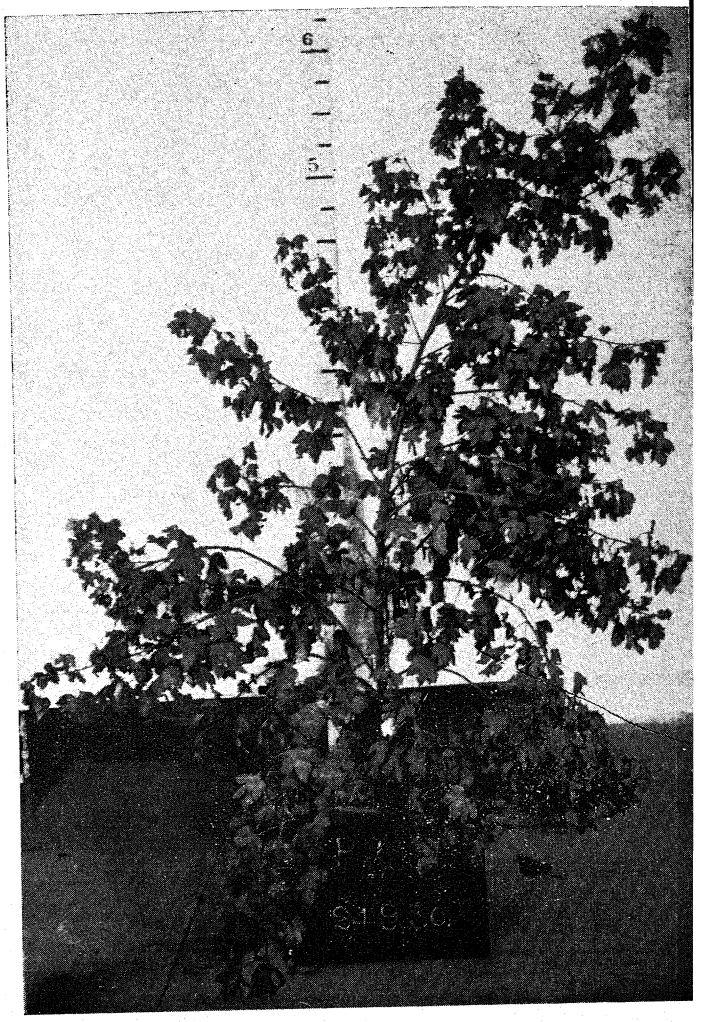
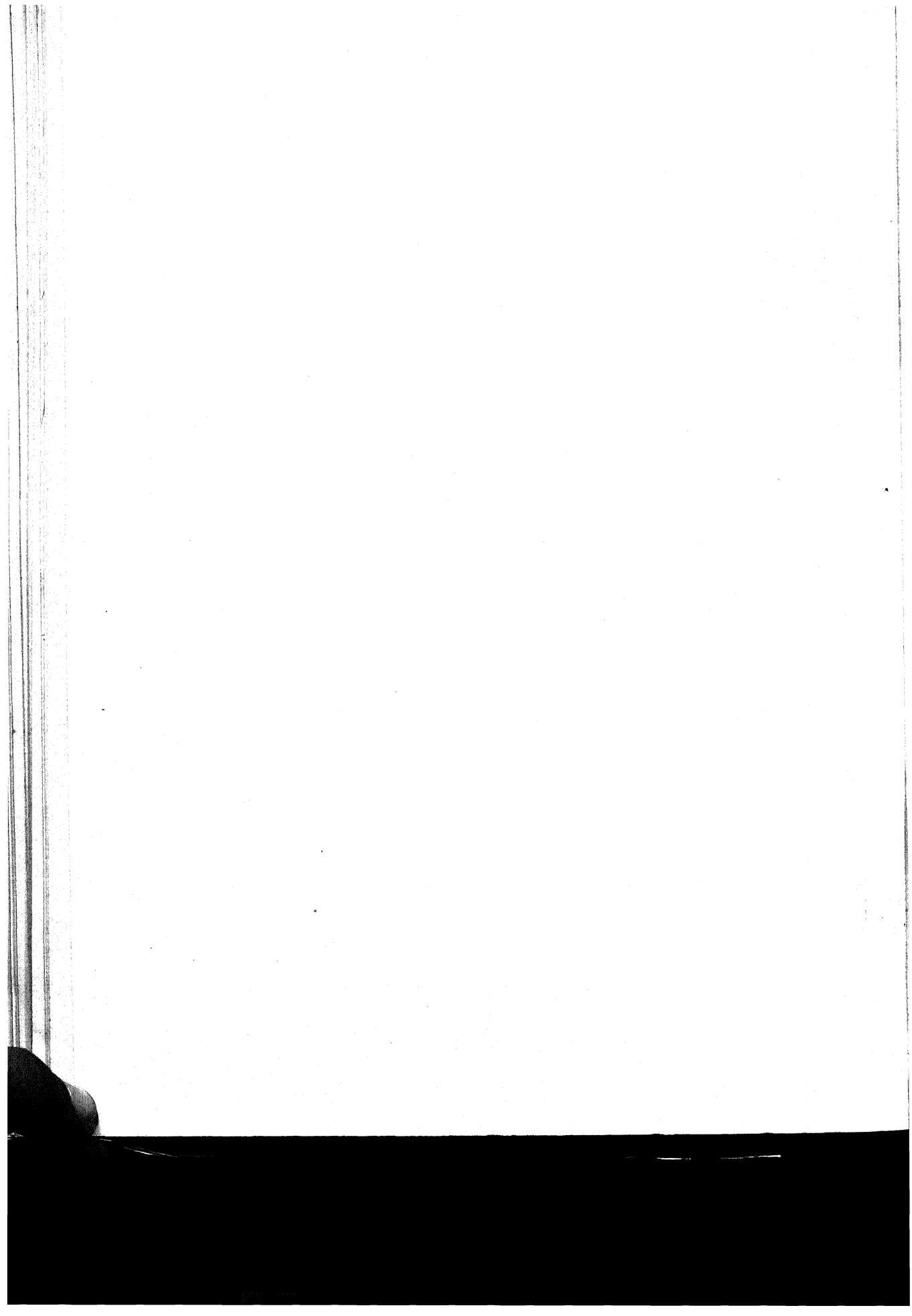


Fig. 2.



in Knop's solution and transplanted in fine compost soil 24 hours afterwards. The embryo could also be directly placed in good soil. Great care is required in peeling off the testa not to injure the embryo.

The plant grows very slowly and rarely grows taller than 18 inches. The flowers are, however, produced during the first season of growth. It is quite hardy and stands ratooning for several years.*

Description of the plant.—A small perennial shrub with thin stem, short internodes with usually a branch at every internode. The monopodial branches are somewhat spreading and fairly long, but the sympodial branches are invariably short. The whole plant has a compact appearance.

Stem hairy, hairs being very short, studded with numerous black dots. The main stem is about one foot high. Leaves small, about 1½ inches long, cordate, usually five-lobed, apex acute or slightly mucronate, inflexed, sinus acute or slightly plicate, leathery texture, very hairy, the hairs being very small and stellate with usually 5 branches, veins prominent with one gland on the midrib,† lamina deep green in colour with many black dots due to resin glands, petiole small, cylindrical and very hairy. Stipules long and persistent. Bracteoles three, absolutely free, with a fairly well defined claw, ovate oblong, margin deeply indented with usually ten teeth, very hairy, hairs being very short, veins fairly prominent. The stipular nectaries mentioned by Watt are absent. Peduncle usually very short and never more than ½ inch long. Calyx cup-shaped with five short teeth, thickly covered with short hairs, pale green in colour. Corolla light yellow in colour, slightly bigger than bracteoles,‡ prominent petal spot at the base, when open the petals are well reflexed,§ pollen light cream in colour. Boll almost spherical with a prominent spine at the apex, black resin glands prominent, 3-locked. Seeds usually 2, but occasionally 3, in each lock, closely compacted together and triangular in section, thickly coated with rusty brown or chocolate coloured fuzz. The seed is very hard and stony.

G. indicum *Gammie* (*Plate XXII, fig. 2*).

A tall plant about 6 feet high with ascending side branches. The monopodial region extends about half-way up the main stem. The monopodia are ascending. The sympodia are fairly long, bearing 4 or 5 flowers.

* One plant in the Cotton Research Laboratory gardens has now been growing for three years and is expected to live for some years more.

† Watt says that the leaves of *G. stocksii* are eglandular.

‡ Watt says the corolla is yellow and twice the size of bracteoles.

§ See also description of *G. stocksii* by Youngman and Pande, *Nature*, Vol. CXXIX, 1927, p. 745. The flowers of *G. stocksii* here described resemble those described by Youngman and Pande rather than those described by Watt.

Stem thickly covered with longish hairs. Leaves big, about 3 inches long, broad-lobed, lobes oblong with acute apex, incision about $\frac{1}{2}$, sinus acute, slightly reflexed, very hairy. Veins prominent with 2-3 glands and numerous black dots, thin in texture and deep green in colour. Stipules small and deciduous. Bracteoles cordate, small, about $\frac{3}{4}$ inch long, highly connate, margin entire, hairy, with a few black dots. The veins are not very prominent. Calyx cup-shaped, with five teeth and a few hairs and with numerous black dots. Corolla bright yellow in colour, fairly big, about $1\frac{1}{2}$ inches long with a big prominent dark purple eye-spot, petals mediumly reflexed when the flower is open. Pollen deep yellow in colour. Bolls quite big, tapering, 3-4 locked, valves recurved when open, 8-11 seeds in each lock. Seeds thickly coated with dirty grey fuzz and profuse white lint.

Hybridization.

Considerable difficulty was experienced in crossing Stocksii with other Indian cottons. During 1928, 21 flowers were crossed; 18 with *G. indicum* Gammie, var. *Mollisoni* as the female parent, and 3 with *G. stocksii* as the female parent, but all the bolls so formed dropped prematurely.

During 1929 it was thought that *G. stocksii*, being a wild cotton from Sind, might cross with a Sind cultivated cotton. For this purpose a plant of *G. indicum* Gammie from a local mixture of Bhitshah cotton was selected. 36 flowers were crossed,* 27 using the *indicum* as the female parent and 9 using the *stocksii* as the female parent and one mature boll from the former was obtained.† This boll was smaller in size than ordinary bolls and contained only four fully developed seeds.

These seeds were sown in the field on 8th May 1930 and all of them germinated in contradistinction to the difficulty of germination found with the *stocksii* parent. These plants proved to be genuine hybrids.

F_1 plants.—Of the four plants reared, three were short, stunted plants looking very much like the *stocksii* parent (Leake's diminutive plants) while the fourth one was a tall, vigorously growing plant. The three short plants have, up to now, not produced any floral buds nor any sympodial branches. The tall plant has, on the other hand, produced numerous flowers which have, however, failed to set bolls. None of the flowers produced were retained for more than five days on the plant. A short description of these plants is appended.

C 15/30 p. 2 (Plate XXIII, fig. 1).—Short plant, about $1\frac{1}{2}$ feet high with many monopodial branches. The secondary branches are all monopodial and up to now

* Mr. Sarup Singh, Senior Technical Assistant, Cotton Research Laboratory, performed these operations.

† During 1930, one crossed boll with *G. stocksii* as the female parent and *G indicum* as the male parent has been obtained. This F_1 will be studied next year.

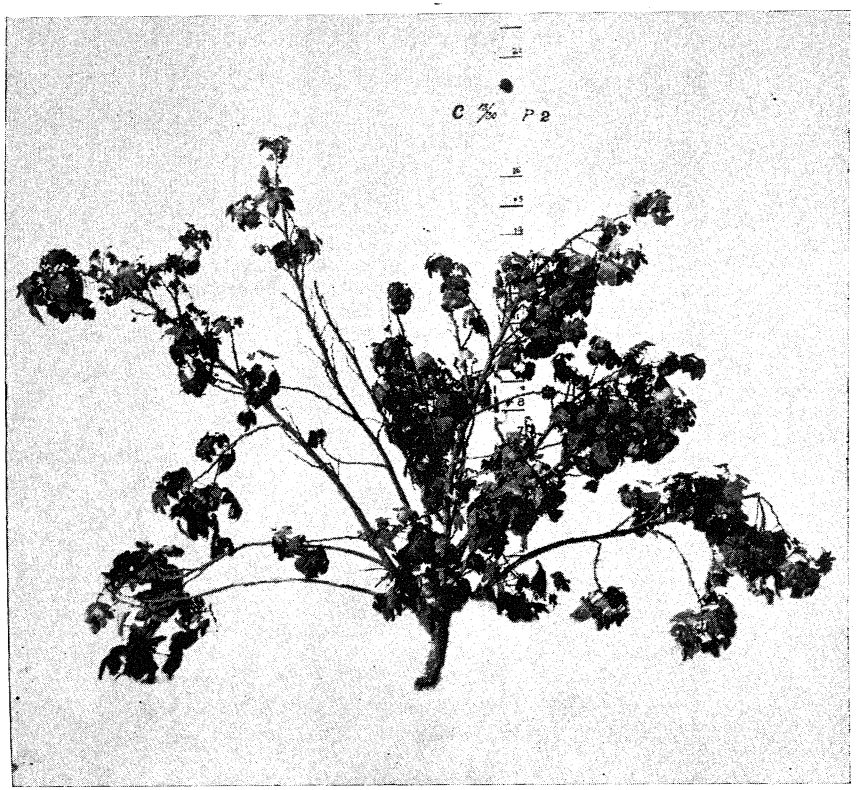


Fig. 1.

Fig. 2.

no sympodial branches have been produced. Stem short and sparsely hairy. Leaves small, slightly more than an inch long, light green in colour, 3-5 lobed, sparsely hairy, lobes ovate, incision about $\frac{1}{2}$, sinus acute, veins prominent with one gland. No flowers were produced. The whole plant has a fairly compact look and resembles *G. stocksii* very much.

C 15/30 p. 1 (Plate XXIII, fig. 2).—A tall plant, about 7 feet high with numerous lateral branches. The laterals up to about 5 feet from the soil are all monopodial. Monopodial branches long with terminal bud capable of making indefinite growth. Some of the lower monopodia measure more than 5 feet. This propensity of the plant to produce several very long monopodial branches gives it a very characteristic rambling appearance. The sympodial branches are, however, very short with usually two buds and very short internodes. Stem—sparsely hairy with short internodes. Leaves—the primary leaves are almost $2\frac{1}{2}$ inches long, but the secondary and tertiary leaves are smaller in size, with branches coming off at practically every leaf-axil, broad lobed, 5-7 lobed, lobes oblong, incision a little more than $\frac{1}{2}$, sinus usually acute or slightly plicate, thin and papery in texture, sparsely hairy, a few short hairs present on the veins. The veins prominent with one or two glands. The leaf is light green in colour and dotted with numerous resin glands. Stipules long and thin and reddish green in colour. Bracteoles small, about $\frac{3}{4}$ inch long, margin indented with 10-12 teeth, fairly hairy, especially on the margin, cordate in shape, non-connate. Calyx campanulate with 5 teeth, light green in colour with numerous black dots. Corolla about $1\frac{1}{4}$ inches long, light yellow in colour, with a prominent dark purple eye-spot of medium size. Pollen light yellow.

A further difference between these two types of F_1 plant emerged during the 1931 season—the three small plants died, while the tall one survived, and grew vigorously.

Thus we can summarize the main characters of the F_1 plants and see how these compare with those of the two parents.

G. indicum Gammie (female parent)	F_1	*G. stocksii* M. Mast (male parent)
(1) Plants tall, about 6 feet high	(1) Three plants small, about $1\frac{1}{2}$ feet high, and one plant about 7 feet high	(1) Plants very small, about 1 foot high
(2) Leaves big, measuring about 3 inches long	(2) The three small plants have leaves measuring about one inch and the one big plant has leaves measuring about $2\frac{1}{2}$ inches	(2) Leaves very small, not more than 1 inch long

G. indicum Gammie (female parent)	F_1	*G. stocksii* M. Mast (male parent)
(3) Leaves thickly hairy, hairs long	(3) All 4 plants with sparsely hairy leaves, hairs very short	(3) Leaves thickly hairy, hairs very short
(4) Two or three glands on the leaves	(4) Usually one gland on the leaves	(4) Usually one gland on the leaves
(5) Epicalyx cordate, with entire margin, highly connate	(5) Epicalyx cordate with dentate margin, non-connate	(5) Epicalyx cordate with deeply indented margin, non-connate
(6) Corolla, big bright deep yellow with big deep purple eye-spot	(6) Corolla, medium in size and colour, with medium deep purple spot (Tall plant only produced flowers)	(6) Corolla, small, light yellow with deep purple small eye-spot
(7) Pollen deep yellow	(7) Pollen light yellow	(7) Pollen cream

Summary.

G. stocksii M. Mast has been successfully crossed with *G. indicum* Gammie. The F_1 plants do not resemble each other, but three plants resemble very much the *stocksii* parent in appearance, while the fourth one is intermediate. No satisfactory explanation has, so far, been found to account for the diversity among the F_1 plants.

All the F_1 plants are sterile.

Acknowledgments.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS.*

No. 9.—CERTAIN VARIETAL STUDIES ON THE COTTON PLANT IN SURAT.

BY

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(Received for publication on 3rd October 1932)

1. Mr. K. V. Joshi, of the Cotton Research Laboratory, Surat, has sent us the data given in Tables I and II showing the percentage success of bolls from flowers for 5 different strains of the cotton plant sown in 1929-30 and 1930-31, respectively.

TABLE I.

(*1929-30*)

——	I	II	III	IV	V
Control	38·2	37·7	38·9	37·9	38·2
Selection A	43·2	41·0	42·3	41·2	40·2
Selection B	46·5	45·3	45·0	45·6	44·7
Selection C	46·8	47·4	49·3	47·1	46·5
Selection D	49·5	46·6	48·7	49·6	47·6

TABLE II.

(*1930-31*)

——	I	II	III	IV	V
Control	38·4	35·9	36·0	35·0	34·4
Selection A	42·9	42·8	37·7	38·8	40·1
Selection B	42·5	44·1	38·4	39·8	40·7
Selection C	48·8	46·3	45·1	44·5	45·8
Selection D	50·4	47·3	47·0	45·5	45·9

* A large number of enquiries of a statistical nature are being received from agricultural workers in different parts of India. Many of these enquiries are of considerable general interest, and it is proposed to publish notes on selected topics from time to time. These notes will deal mainly with statistical methods and procedure, and it is not intended that they should always contain new matter.

2. Fisher's method of analysis of variance has been used for studying the above results. The value of observed z is given in each case by :—

$$z=\frac{1}{2}\log_e v/v_0$$

where v_0=residual variance (error). The 5 per cent. (or one per cent.) values of z have been taken from Fisher's Table VI.

The analysis for 1929-30 is given in Table III.

TABLE III.

(1929-30)

—	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	5 per cent.
Control *vs.* Selection	1	226·80	226·80	2·8903	0·7514
Selections	3	135·69	45·23	2·0793	0·5876
Varieties	4	362·49	90·62	..	..
Soil differences	4	8·95	2·24	..	..
Error	16	11·13	0·696	..	..
	24	382·57			

The varietal differences in yield are very pronounced and statistically significant.

The mean percentage of success and the respective differences are shown in Table IV.

TABLE IV.

(1929-30)

—	Mean percentage of success	Difference from				
		Control	Selec. 43	Selec. 65	Selec. 32	Selec. 9
Control	38·18	..	—3·42	—7·24	—9·24	—10·22
Selection A . . .	41·60	3·42	..	—3·82	—5·82	—6·80
Selection B . . .	45·42	7·24	3·82	..	—2·00	—2·98
Selection C . . .	47·42	9·24	5·82	2·00	..	—0·98
Selection D . . .	48·40	10·22	6·80	2·98	0·98	..

Standard error of difference in means=0·53.

We find :—

(*i*) Compared to control, all the selected varieties exhibit significantly higher percentage success of boll-formation from flowers.

(*ii*) Compared to Selection A, the three other varieties Selections B, C and D are definitely superior.

(*iii*) Selections D and C appear to be better than Selection B.

(*iv*) The difference in mean percentage of success of boll-formation from flowers between Selections D and C is inappreciable.

3. The analysis for the data for 1930-31 is shown in Tables V and VI.

TABLE V.
(*1930-31*)

——	D. F.	Sum of squares	Mean variance	Value of *z*	
				Observed	5 per cent.
Control *vs.* Selections	1	242·12	242·12	2·6578	0·7514
Selections	3	177·05	59·02	1·9519	0·5876
Varieties	4	419·17	104·79	..	..
Soil differences	4	58·16	14·54	..	..
Error	16	19·01	1·10	..	..
	24	496·34			

The mean percentages and differences are given in Table VI.

TABLE VI.
(*1930-31*)

——	Mean percentage of success	Difference from				
		Control	Selec. 43	Selec. 65	Selec. 32	Selec. 9
Control	35·9	..	—4·6	—5·2	—10·2	—11·3
Selection A . . .	40·5	+4·6	..	—0·6	—5·6	—6·7
Selection B . . .	41·1	+5·2	+0·6	..	—5·0	—6·1
Selection C . . .	46·1	+10·2	+5·6	+5·0	..	—1·1
Selection D . . .	47·2	+11·3	+6·7	+6·1	+1·1	..

Standard error of difference in mean = 0·69.

We again observe that:—

(*i*) All the selected strains are significantly better than the control.

(*ii*) Selections D and C are better than Selections A and B.

(*iii*) The difference between Selections A and B, as also the difference between Selections D and C are not significant.

(*iv*) Finally, the two years' data may be combined when the analysis takes a slightly different form.

TABLE VII.

(1929-30 and 1930-31)

—	D. F.	Sum of squares	Mean variance	Value of z	
				Observed	5 per cent.
Control *vs.* Selections	1	468·80	468·86	1·9214	0·7514
Selections	3	294·57	98·19	1·1397	0·5876
Varieties	4	763·43	190·86	..	..
Soil differences	8	67·11	8·29	..	..
Seasons	1	52·02	52·02	1·8221	0·7072
Error	36	48·43	1·36	..	..
	49	930·99			

TABLE VIII.

(1929-30 and 1930-31)

—	Mean percentage of success	Difference from				
		Control	Selec. 43	Selec. 65	Selec. 32	Selec. 9
Control	37·06	..	—3·97	—6·20	—9·70	—10·75
Selection A . . .	41·03	+3·97	..	—2·23	—5·73	—6·78
Selection B . . .	43·26	+6·20	+2·23	..	—3·50	—4·55
Selection C . . .	46·76	+9·70	+5·73	+3·50	..	—1·05
Selection D . . .	47·81	+10·75	+6·78	+4·55	+1·05	..

Standard error of difference in mean = 0·51 or 1·2 per cent. of mean value.

We find that—

(*a*) The superiority of—

(*i*) All the selections over the Control,

(*ii*) Selections B, C and D over Selection A,

(*iii*) Selections C and D over Selection B

is clearly established.

(*b*) The difference between Selections C and D is on the verge of significance.

(*c*) The seasonal difference is clearly significant.

5. The performance of Selection A was the subject of specific inquiry. The data for several seasons for control and Selection A are given in Table IX.

TABLE IX.

Season	Variety	No. of flowers	No. of bolls	Percentage success of bolls
1924-25	Control	84·3	34·9	41·4
	Selection A	68·4	25·5	37·3
1925-26	Control	87·4	30·0	34·3
	Selection A	92·9	32·8	35·3
1926-27	Control	66·5	24·1	36·3
	Selection A	70·6	29·6	41·9
1927-28	Control	87·0	35·5	40·8
	Selection A	85·2	36·7	43·1
1928-29	Control	90·8	34·3	37·7
	Selection A	89·0	35·9	40·3
1929-30	Control	55·6	21·2	38·1
	Selection A	61·9	26·7	43·2
1930-31	Control	69·3	24·3	35·0
	Selection A	65·2	25·4	38·9

A direct difference method for comparison may be adopted with advantage. The differences between Selection A and the control are shown separately for flowers, bolls and percentage success for each season in Table X.

TABLE X.

Season	Flowers	Bolls	Percentage success
1924-25	—15·9	—9·4	—4·1
1925-26	+5·5	+2·8	+1·0
1926-27	+4·1	+5·5	+5·6
1927-28	—1·8	+1·2	+2·3
1928-29	—1·8	+1·6	+2·6
1929-30	+6·3	+5·5	+5·1
1930-31	—4·1	+1·1	+3·9

We can now find the value of the standard deviation of each set of differences and obtain the values of z given in Table XI. The probability of occurrence of the observed value of z in each case is next found from Table XXV of the Tables for Statisticians and Biometricians, Part I.

TABLE XI.

——	Mean diff.	S. D.	z	P	Odds (approximate)
Flowers	—1·1	7·12	—·15	0·6276	2 : 3
Bolls	+1·18	4·66	+·25	0·7174	3 : 1
Percentage success of flowers to bolls	2·63	3·02	+·87	0·9610	24 : 1

Selection A is thus significantly superior to the control so far as the percentage success of flowers to bolls is concerned ; but the difference in the number of flowers or the number of bolls per plant is insignificant.

It is worth noting that the precision of the comparison is 1·2 per cent. which compares favourably with the highest degree of precision reached in varietal trials anywhere else.

We would like to add that this note is written purely from a statistical standpoint. Details of the experiments with full discussion of their agricultural bearing will be published in due course by Mr. Joshi himself.

NO. 10—THE ANALYSIS OF A MANURIAL EXPERIMENT ON WHEAT CONDUCTED AT SAKRAND, SIND.

BY

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Mr. V. A. Tamhane, the Agricultural Chemist and Soil Physicist in charge of Agricultural Research Station, Sakrand, Sind, conducted a manurial experiment on wheat with five different treatments, replicated 8 times. The experiment was conducted on a block of two acres and a half, divided into 40 plots of $\frac{1}{16}$ acre each. Owing to a change of programme, the number of plots available for analysis for treatment A (no manure) was 5, while for the other 4 manurial treatments was 8 each.

The experimental arrangement is shown in Fig. 1. The plots marked "spoiled" had a dose of compost and cannot, therefore, be considered untreated. It will be noticed that originally all the 5 treatments had been distributed once in each column. But in columns 2, 4 and 8, treatment A was spoiled by the addition of compost, thus rendering it impossible to compare these columns directly with the rest.

Rows ↓ / Cols. →	1	2	3	4	5	6	7	8
1	E 51	C 1 54	D 58	B 37	D 48	A 44	E 59	C 58
2	D 46·5	B 52	A 42	C 52	E 3 60	C 50	D 4 50	A(spoiled) 45·5
3	C 48	A(spoiled) 60	E 82	D 2 53	A(spoiled) 51	B 43·5	A 52	B 49·25
4	B 5 44·25	D 50	B 6 35	A 36	C 45	E 7 51	C 49	E 8 50
5	A 17·25	E 34	C 27	E 22	B 41	D 52	B 30	D 24

FIG. 1.

The experimenter himself divided the field into an extremely artificial system of blocks shown in thick lines in Fig. 1. It is not clear why this was done, unless of course results of previous uniformity trials had definitely indicated the usefulness of such a division.

2. The present analysis has, however, been made both with the experimenter's own type of block-division, as well as with a straightforward columnar division. In the first instance blocks with the spoiled plots were left out.

(*a*) The analysis according to columnar division is shown in Table I.

TABLE I.

	D. F.	Sum of squares	Mean square	Value of z	
				Observed	5 per cent.
Between treatment . . .	4	995·09	248·77	0·2201	·5265
Block . . .	4	353·36	88·34		
Error . . .	16	2,849·98	178·12		
Within treatment . . .	20	3,203·34	160·17		
		4,198·43			

The observed z is considerably lower than the 5 per cent. point, and therefore the treatment differences cannot be considered significant. The differences between the 5 blocks also appear to be insignificant and thus the columnar system of block division has been ineffective in enhancing the precision of the experiment. The "block" and "error" variances were, therefore, combined, and the residual variance was calculated for a larger number of degrees of freedom.

3. (*b*) The experimenter's system of block division gave the following analysis :—

TABLE II.

	D. F.	Sum of squares	Mean square
Treatment	4	192·63	48·16
Blocks	4	1,086·60	271·65
Error	16	1,912·64	119·54

The variance for treatment is smaller than the residual variance, indicating that the treatment differences are not significant.

4. The experimental data may be studied from another standpoint. We have here studied the yields for 5 plots for treatment A and for 8 plots for the other 4 treatments. Assuming that these yields are independent measures of the mean yield of the 5 treatments, we obtain the following analysis for Fisher's t-test.

TABLE III.

—	Degrees of freedom	Sum of squares	Mean square	Variance of mean
A . . .	4	682·25	170·56	34·11
B . . .	7	492·87	70·41	8·80
C . . .	7	607·00	86·71	10·84
D . . .	7	726·47	103·78	12·97
E . . .	7	2,236·87	319·75	39·97

TABLE IV.

—	Mean diff.	Variance of mean diff.	S. E. of mean diff.	t	n	P greater than
A—B . . .	2·70	34·72	5·89	0·46	11	0·6
A—C . . .	7·35	38·09	6·17	1·19	11	0·2
A—D . . .	7·05	41·62	6·45	1·10	11	0·2
A—E . . .	5·15	86·25	9·29	0·54	11	0·6
B—C . . .	6·38	19·67	4·44	1·44	14	0·1
B—D . . .	6·19	21·77	4·67	1·33	14	0·2
B—E . . .	9·63	48·75	6·98	1·38	14	0·1
C—D . . .	7·72	23·81	4·88	1·58	14	0·1
C—E . . .	7·93	50·79	7·13	1·11	14	0·2
D—E . . .	12·15	52·92	7·28	1·67	14	0·1

The probability of occurrence of "t" is obtained in each case from Fisher's Table IV [1930].

If we work with the conventional value $P=·05$ as the level of significance, the last column of the Table IV definitely indicates that none of the differences reached

the required level ; it would not, therefore, be possible to draw any positive conclusion from these experimental data.*

5. In conclusion, a few words on the principles underlying the division of the field into " blocks " may prove useful. The factors producing differences in soil fertility may be divided broadly into two groups : (*a*) systematic changes in fertility from one part of the field to another, and (*b*) chance fluctuations which are distributed in a random manner all over the field. The purpose of division into blocks is to eliminate the systematic changes, while the purpose of replication of plots within blocks is to furnish a reliable estimate of the random fluctuations. The blocks should then be arranged in such a way as to include within each block an appreciable portion of the systematic variation in fertility.

Consider a square or rectangular field with sides running in a north-south and an east-west directions. If we know that the systematic variation in fertility occurs only in one particular direction, say from north to south, then it will be necessary to use block divisions only in this particular direction. It is clear that block divisions in a perpendicular (that is east-west direction) will not show any systematic change in fertility, and hence will be of no use in eliminating effects due to soil heterogeneity. Now suppose that we have no information available regarding the direction of change of the systematic variation in fertility. It will now be obviously desirable to provide blocks in two directions at right angles. Generally speaking, information regarding soil heterogeneity is not available beforehand. This is why we usually provide block divisions in two directions at right angles so that systematic fluctuations in fertility along two perpendicular directions may be simultaneously eliminated. Fisher's " Randomized Block " and " Latin Square " (in which the number of blocks is same in each direction) are typical examples of arrangements based on this principle.

In case, however, previous knowledge regarding the distribution of fertility of the soil is available from uniformity trials, it is possible and it may be desirable to make special arrangements of the blocks so as to eliminate the effects of soil heterogeneity in the most effective manner.

* The manurial treatments used were :—

A=Control : untreated

B=Half usual organics (7½ cart loads of compost)

C = „ *plus* sulphate of ammonia @ 10 lbs. nitrogen per acre

D= „ „ @ 20 lbs. „

E= „ „ @ 30 lbs. „

Variety : Pusa 12. *Sowing dates* : 23/24 December, 19..1.

NO. 11—THE USE OF THE METHOD OF PAIRED DIFFERENCES FOR ESTIMATING THE SIGNIFICANCE OF FIELD TRIALS.

BY

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Very recently we received a paper for criticism in which the author used the method of paired differences for estimating the significance of field trials. The use of this method is justified only in special cases, and a discussion of the principles involved may prove useful to field workers.

Consider a hypothetical experimental field which is completely free from systematic variation in fertility. Suppose we have "$2n$" plots of which "n" are sown with each of the treatments (or variates) A and B. Let $x_1, x_2, x_3 \ldots\ldots x_n$ be the yield of the "n" plots under A, and $y_1, y_2, y_3 \ldots\ldots y_n$ for the "n" plots under B. Let $d_1=(x_1-y_1)$, $d_2=(x_2-y_2)$.....etc., be the difference of yield for paired plots of A and B. The observed mean value of the difference $\bar{d}=(\bar{x}-\bar{y})$, where $\bar{x}$ and $\bar{y}$ are the mean yields of A and B. The observed value $\bar{d}$ is clearly equal to the real difference in yield between A and B *plus* the experimental errors, since by hypothesis there are no systematic differences in fertility between different plots. We may, therefore, proceed to calculate in the usual way s_d, the variance of the difference, and use it to judge whether $\bar{d}$ is significantly different from zero or not. Fisher's t-test with

$$t=\frac{\bar{d}\sqrt{n}}{s_d} \qquad \ldots\ldots\ldots \qquad (1)$$

and $(n-1)$ degrees of freedom can then be applied in the usual way.

It is clear that in this method we can pair x and y in any way we like. For example, we can take $d_1=x_1-y_4$, or $d_2=x_{12}-y_5$, etc. The mean value of d and the standard deviation s_d would not be sensibly affected by the method of pairing (since x and y are supposed to be quite independent), and hence the value of "t" will remain the same.

2. The conditions under which the method of analysis of variance is used are entirely different. Systematic changes in fertility cannot be assumed to be absent (and, in fact, are usually known to be present), and hence the differences in yield between any two plots will definitely include the effect of differences in soil fertility.

In this case we first try to allow for the effects of soil heterogeneity by the elimination of the variance " between blocks", and then judge the significance of the effect of treatments (or varieties) in the usual way with the help of Fisher's z-test.

3. Where systematic differences in soil fertility are present (or cannot be assumed to be absent) it is clear that we cannot legitimately use the method of paired differences. Soil heterogeneity will almost never be entirely absent, and hence the necessary conditions for the use of paired differences will never be strictly fulfilled in practice. But under certain circumstances we may reasonably assume the effect of extraneous factors to be either absent or to be appreciably constant in magnitude. For example, consider pairs of adjacent plots sown with two different varieties. In ordinary circumstance (that is, unless changes in fertility are very sharp) we may, as a first approximation, assume that the soil fertility will remain nearly the same for each pair of adjacent plots. Hence if we take the difference in yields from two adjacent plots, we may be reasonably certain that the effect due to the soil factors will be eliminated in the process of differencing. Under these circumstances, that is, when the differences in yield refer to adjacent plots, the use of paired differences may possibly be justified.

But the method cannot obviously be extended to the case of plots which are not adjacent. Consider x_1 the yield of A from Plot No. 1, and y_{16} the yield of B from Plot No. 16 which is situated at a considerable distance from Plot No. 1. The difference in yield ($d=x_1-y_{16}$) must obviously include not only the effect due to the varietal difference between A and B and the residual errors, but also the effect due to differences in soil fertility from one part of the field to another. The use of the method of paired differences in these circumstances has, therefore, absolutely no justification.

4. A numerical example may make the position clear. In a manurial experiment on wheat, two treatments A and B were laid out in a Randomized Block in 8 replications, and the yields obtained (in lbs. per 1/40th acre) were as shown in Table I. The serial number of the plot is shown against each plot.

TABLE I.

1	2	3	4	5	6	7	8
A	B	B	A	A	B	A	B
47·0	68·5	62·0	55·0	62·5	73·5	56·0	66·0
B	B	A	B	A	A	B	A
63·5	61·0	44·5	57·0	66·0	52·0	34·0	38·0
9	10	11	12	13	14	15	16

Using the usual method of analysis we obtain the mean value of yield under A=52·64, and the mean value of the yield under B=60·69, so that the difference in mean yield is 8·05. The standard error of the difference is 5·23, so that t=1·54. From Fisher's Table IV we find that with n=7, the probability of occurrence lies between 0·1 and 0·2. So that even if the difference between A and B is *nil*, the observed difference would occur in from 10 per cent. to 20 per cent. of cases. The difference between the two treatments cannot therefore be considered significant.

Now let us try to use the method of paired difference. We choose the pairs as shown in Table II.

TABLE II.

Treatment B		Treatment A		Difference in yield
Plot No.	Yield	Plot No.	Yield	
6	73·5	13	66·0	7·5
2	68·5	5	62·5	6·5
8	66·0	7	56·0	10·0
9	63·5	4	55·0	8·5
3	62·0	14	52·0	10·0
10	61·0	1	47·0	14·0
12	57·0	11	44·5	12·5
15	34·0	16	38·0	—4·0

Mean difference = 8·05
Standard error of difference = 2·018

$$\therefore t = \frac{8·05}{2·18}$$

$$= 3·994$$

$$P < 0·01$$

The probability of occurrence is now less than 0·01, so that on the results of the present mode of analysis the difference between treatments A and B would be considered to be definitely established. But there is no justification for choosing the pairs in this special way, and hence the estimate of the standard error is wholly invalid.

In fact the value of the standard error depends entirely on the particular manner in which the plots are paired. Consider for example the following system of pairing (Table III).

TABLE III.

Treatment B		Treatment A		Difference in yield
Plot No.	Yield	Plot No.	Yield	
6	73·5	16	38·0	35·5
2	68·5	11	44·5	24·0
8	66·0	1	47·0	19·0
9	63·5	14	55·0	8·5
3	62·0	4	52·0	10·0
10	61·0	7	56·0	5·0
12	57·0	5	62·5	—5·5
15	34·0	13	66·0	—32·0

Mean difference = 8·05

Standard error of difference = 7·23

$$\therefore t = \frac{8·05}{7·23}$$

$$= 1·11$$

$$P > 0·3$$

The value of t is now only 1·11, and the probability of occurrence is greater than 0·3. On the result of this analysis the effect of both treatments would appear to be statistically indistinguishable.

We can pair the plots in a large number of different ways, and each particular way of pairing would lead to a different value of the "standard error", and hence to a different value of t. But all such values are equally invalid, and no legitimate inference can be drawn from any arbitrarily-paired system of differences.

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THE COMPOSITION OF THE RAIN WATER OF SYLHET.

BY

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Analysis of the dissolved constituents of rain water has been made in different parts of the world from the early eighties of the nineteenth century. The idea underlying has always been to find out how much of combined nitrogen, either as ammonia or as nitric nitrogen, is furnished to the soil in different countries by the rain water and also if there is any difference in the proportion of nitric nitrogen as compared to ammonia in the tropical and non-tropical countries. The halogen content has also been determined in certain places as also the dissolved solids.

At Rothamsted and at a few other places records have been kept for a good many years for purposes of comparison. The most notable works in this connection are those of Lawes, Gilbert and Warington [1883], Miller [1905] and Russel and Richards [1919].

Miller, in his fairly exhaustive summary of the results of analysis of the rain water of 32 different places in tropical and non-tropical countries, noticed considerable variation regarding the total nitrogen in similarly situated places, which he found difficult to explain. He thus concluded that great difference in climate was not coincident with the amounts of nitrogen brought down by rain. Generally speaking he noticed that in non-tropical countries the proportion of ammonia was higher while in the tropics that of nitric nitrogen was in excess. But even then this regularity has been far from perfect and he therefore remarked that further analysis was desirable as might throw light on any difference in the composition of rain, due to the condition of the winds.

Since then the analysis of rain water has been done in several places, such as Cawnpore and Dehra Dun in India (1906), Lincoln in Newzealand (1907-1909), Garforth in Leeds (1909), Flahult in Sweden (1910), Barbados (1910), Queensland (1910), Tonquin in Malaya (1911), British Guiana (1911), Mount Vernom in Iowa (1914), Durban and in South Africa (1914), Ottawa (for 10 consecutive years), etc., etc.

Russel and Richards [1919] made a summary of the Rothamsted records and observed that of the various sources of ammonia (sea, soil and city pollution) the soil happened to be the most important and further that the ammonia content was higher during the period of high biochemical activity and low during the period of low biochemical activity. They further noticed a close relationship between ammonia and nitric nitrogen and suggested that there was either a common origin or that the nitric compounds were formed from ammonia. They also noticed a difference in the composition of the summer and winter rain and concluded that they were possibly of different origin.

In India almost the only complete systematic analysis of the composition of rain water is that of Leather [1906] with regard to the rainfall of Dehra Dun and Cawnpore (one situated in N. Lat. 30, E. Long. 78 and the other in N. Lat. 27 and E. Long. 80). Isolated samples seemed to have been analysed at Madras, Calcutta and Ceylon.

Leather concluded, from the results of his analysis, that the total quantity of nitrogen carried by the rain to the soil was approximately equal to that in the rain at Rothamsted. (Dehra Dun :—3·40, Cawnpore :—3·25, Rothamsted :—3·84 pounds per acre); but that the relative amounts of nitric nitrogen was higher at Dehra Dun and lower at Cawnpore than at Rothamsted. He tried to explain the difference by the difference in the altitude as also by the fact that the rains of Dehra Dun were more frequently accompanied by thunderstorms than that of Cawnpore.

Cherrapunji, in the Khasi Hills (Assam, India), has the reputation of the highest rainfall in the world. The summer monsoon is responsible for it. An average of 28 years showed a rainfall of 457·80 inches of which 428·13 inches fell between the months of April and September.

According to Dr. Hooker, this unparalleled amount of rainfall was attributable to the abruptness of the mountains which face the Bay of Bengal, from which they were separated by 200 miles of 'jheels' and 'Sunderbands'.

The South-West monsoon (summer monsoon) which is very powerful in India* carries with it an abundant quantity of warm and moist air of the nearly equatorial winds blowing across the Bay of Bengal and these are concentrated by the converging coasts on the highlands on the east and west before they commence their ascent up to mountain slopes and having previously lost little or no vapour by condensation, the whole vapour is now condensed in the ascent of the Himalayan slope.

Sylhet is situated in N. Lat. 25, E. Long. 92, about 25 miles south of Khasi Hills on which stands Cherrapunji, and 175 miles from Bay of Bengal. The summer

*'In no other parts of the world are the conditions of a powerful monsoon influence so favourable and the strength of the monsoon produced so great as in India and the North Indian Ocean.' (Ferrel, Winds, P. 200-201.)

monsoon which generally sets in April and lasts till September and whose direction is South-West must go past Sylhet to reach Cherrapunji. Hence the analysis of the rain water of Sylhet was thought to be interesting. Besides, the type of clouds which are generally noticed during Sylhet rains is Cumulo-nimbus and these are frequently associated with thunderstorms.

Sylhet is a small town of a population of about 17,000 with no industries worth the name. The locality is rather marshy and the population scattered. During the rains large tracts of land go under water which rise to the surface with the subsidence of flood. The temperature varies between 25° to 22°C. The rainfall is heavy, average being about 150 inches a year.

The sampling and the analysis of the rain was done in the college compound which is about a couple of miles from the town proper.

A rain gauge was installed in a suitable place within the college compound and samples of a week or more were mixed in proportionate quantities and analysed at the earliest opportunities. The samples were analysed for (*a*) free ammonia, (*b*) albuminoid ammonia, (*c*) nitric nitrogen and (*d*) nitrous nitrogen.

Free ammonia was estimated by boiling the requisite quantities with ignited soda carbonate and then Nesslerizing the distilled product, albuminoid ammonia by boiling the residue with alkaline permanganate and Nesslerizing the nitrate by the phenol-sulphonic method and nitrite by the 'Griess-Illosvoy' method.

TABLE I.

Nitrogen as ammonia, nitrate and nitrite.

1931-32	Rainfall in inches	NITROGEN								Albuminoid ammonia	
		Parts per million				Pound per acre					
		As free ammonia	As nitrate	As nitrite	Total nitrogen	As free ammonia	As nitrate	As nitrite	Total nitrogen	Parts per million	Lb. per acre
8th to 14th April, 1931	1·9	0·798	0·237	0·059	1·094	0·343	0·102	0·025	0·470	0·609	0·262
15th to 21st April, 1931	7·49	0·404	0·050	0·041	0·495	0·683	0·085	0·069	0·837	0·428	0·725
22nd to 28th April, 1931	10·73	0·264	0·083	0·044	0·391	0·639	0·202	0·107	0·948	0·395	0·959
29th April to 5th May, 1931	10·38	0·017	0·091	0·041	0·149	0·039	0·214	0·095	0·348	0·124	0·290
6th to 12th May, 1931	10·54	0·025	0·082	0·030	0·137	0·059	0·195	0·070	0·324	0·148	0·353

TABLE I—*contd.*

Nitrogen as ammonia, nitrate and nitrite—contd.

1931-32	Rainfall in inches	NITROGEN									
		PARTS PER MILLION				POUND PER ACRE				ALBUMINOID AMMONIA	
		As free ammonia	As nitrate	As nitrite	Total nitrogen	As free ammonia	As nitrate	As nitrite	Total nitrogen	Parts per million	Lb. per acre
13th to 19th May, 1931	4·24	0·132	0·115	0·041	0·288	0·126	0·110	0·039	0·275	0·124	0·118
20th May to 2nd June, 1931	11·37	0·025	0·178	*Nil*	0·203	0·065	0·458	*Nil*	0·523	0·128	0·328
3rd to 16th June, 1931.	9·83	*Nil*	0·091	*Nil*	0·091	*Nil*	0·202	*Nil*	0·202	0·078	0·174
17th to 30th June, 1931	19·5	0·136	0·044	*Nil*	0·180	0·600	0·191	*Nil*	0·791	0·148	0·653
1st to 14th July, 1931	7·38	0·300	0·045	*Nil*	0·345	0·500	0·074	*Nil*	0·574	0·115	0·192
15th to 27th July, 1931	7·08	0·082	0·040	*Nil*	0·122	0·131	0·064	*Nil*	0·195	0·107	0·171
28th July to 11th August, 1931	11·45	0·049	0·091	*Nil*	0·140	0·128	0·235	*Nil*	0·363	0·037	0·096
12th to 25th August, 1931	5·18	0·078	0·040	*Nil*	0·118	0·091	0·047	*Nil*	0·138	0·078	0·091
26th August to 8th September, 1931	12·39	0·033	0·066	*Nil*	0·099	0·092	0·185	*Nil*	0·277	0·165	0·461
9th to 22nd September, 1931	8·29	0·087	0·125	*Nil*	0·212	0·162	0·234	*Nil*	0·396	0·029	0·054
23rd September to 6th October, 1931	3·84	0·041	0·100	*Nil*	0·141	0·036	0·087	*Nil*	0·123	0·029	0·025
7th to 20th October, 1931	3·19	0·008	0·166	*Nil*	0·174	0·006	0·120	*Nil*	0·126	0·033	0·024
21st October to 3rd November, 1931	4·81	0·041	0·050	*Nil*	0·091	0·045	0·054	*Nil*	0·099	0·134	0·146
4th to 17th November, 1931	1·92	0·049	0·153	*Nil*	0·202	0·021	0·066	*Nil*	0·087	0·231	0·100
21st January to 11th February, 1932	1·25	1·054	0·352	0·007	1·413	0·298	0·100	0·002	0·400	0·309	0·087
12th February to 23rd March, 1932	2·29	0·642	0·357	0·015	1·014	0·332	0·185	0·008	0·525	0·132	0·068
24th March to 7th April, 1932	0·59	1·029	1·000	*Nil*	2·029	0·137	0·133	*Nil*	0·270	0·208	0·028
TOTAL .	155·64	5·294	3·556	0·278	9·128	4·533	3·343	0·415	8·291	3·789	5·405

Table II shows how the Sylhet rain compares with that of Dehra Dun, Cawnpore and of Rothamsted.

TABLE II.

Comparison of rain at Sylhet, Dehra Dun, Cawnpore and Rothamsted.

Station	Rainfall	Nitrogen, pounds per acre		Total	Ratio $NH_3 : NO_3$
		As ammonia	As nitrate and nitrite		
Sylhet . . .	155·64	4·533	3·757	8·290	1 : 0·82
Dehra Dun . .	86·48	2·037	1·368	3·405	1 : 0·67
Cawnpore . .	49·36	2·482	0·768	3·250	1 : 0·31
Rothamsted . .	27·25	2·712	1·128	3·840	1 : 0·42

The total quantity of combined nitrogen is much in excess of that of the other two Indian stations as also of Rothamsted. The percentage of ammonia to nitric nitrogen is also much higher.

The sources of ammonia are believed to be (*a*) the sea, (*b*) the soil and (*c*) city pollution.

Sylhet is separated from the Bay of Bengal by a distance of about 175 miles and in between lies the Sunderbands and parts of Eastern Bengal. A part of the water vapour carried from the sea is condensed and precipitated on the way and some of the 'sea ammonia' should go down with it. Yet, as the major part of the vapour is deposited at Cherrapunji and its neighbourhood, appreciable amount of sea ammonia must have been brought with it ; but the main supply of ammonia must have been due to the biochemical activity on the marshy lands under the tropical sun. As has been pointed out before, vast tracts lie submerged during the rains and are gradually exposed to the sun with the subsidence of this water. This also perhaps explains the fairly large amount of albuminoid ammonia in the rain water of Sylhet.

It will be seen that during the months of July to October, when the land is mostly under water, the ammonia-content is rather low (1·189 lbs.), while from January to April, it is fairly high (3·472 lbs.).

The sources of nitric nitrogen are (*a*) lightning discharges and (*b*) the oxidation of ammonia by the atmospheric oxygen under electric disturbances and also by ozone or hydrogen peroxide, both of which increase with electric disturbances of the atmosphere.

According to the current theory, the tropical rain should have a higher percentage of nitric nitrogen due to more frequent electric disturbances ; again the lighter ammonia, whose proportion has been found fairly large and which should rise to the heights where clouds are formed, should contribute a portion of nitric nitrogen at its expense, as explained in the previous paragraph.

What per cent. may really come from the direct oxidation of the atmospheric nitrogen by the electric disturbances at the rarefaction as exists at high altitude and whether or not major part comes from the oxidation of ammonia, is a question which we are not in a position to answer. But the remarks of Russel and Richards (*loc. cit.*) in this connection should be interesting. Unless the atmospheric nitrogen is directly oxidised, the contribution of the total combined nitrogen in the atmospheric air should be from ammonia and through ammonia.

In any case the amount of nitric nitrogen is much greater in the Sylhet rain than in the other two Indian stations and in Rothamsted, and the percentage also much higher than in the above stations.

A table has been drawn up of the results of analysis of the rain water of stations more in a line with Sylhet, *i.e.*, which are either in the tropics or very nearly so.

TABLE III.

Analysis of the rain water.

Station	Rainfall	NITROGEN (POUNDS PER ACRE)		Total	Ratio of ammonia to nitric nitrogen
		As ammonia	As nitrate and nitrite		
Barbados . .	59·40	1·009	2·443	3·452	1 : 2·42
British Guiana	106·71	1·321	2·190	3·511	1 : 1·66
Queensland (Brisbane)	45·44	2·228	1·920	4·148	1 : 0·86
Queensland (Cairns)	75·15	1·355	1·766	3·131	1 : 1·30
Lincoln (New-zealand).	29·70	0·513	1·198	1·711	1 : 2·33
Dehra Dun . .	86·48	2·037	1·368	3·405	1 : 0·67
Tonquin (Malaya) .	57·91	4·390	3·540	7·930	1 : 0·80
Venezuela . .	..	14·300	..	..	..
Mauritius . .	..	..	6·340	..	..
Sylhet . . .	155·05	4·533	3·757	8·290	1 : 0·82
Pretoria . .	24·31	6·587	1·083	7·670	1 : 0·16
Durban . .	42·34	3·651	1·234	4·884	1 : 0·34
Cawnpore . .	49·36	2·482	0·768	3·250	1 : 0·31
Rothamsted* .	27·25	2·710	1·130	3·840	1 : 0·43

*Rothamsted figures are given for purposes of comparison.

It will be seen that there is considerable variation in the total amount of nitrogen brought down by rain. We are afraid the statement that ' the tropical rain does not supply to the soil an essentially greater amount of nitrogen than the rain of the temperate climates' [Miller, 1905] has to be accepted with certain reservations. The totals of Pretoria, Tonquin, Venezuela (ammonia alone), Mauritius (nitric nitrogen alone), and Sylhet are results to the point.

With regard to the percentage of ammonia to nitric nitrogen, it would be seen that with the exception of Pretoria, Durban and Cawnpore there is practically a regularity in the higher percentage of nitric nitrogen to that of ammonia in the tropics.

Summary.

1. Rainfall at Sylhet in the year under review had been 155 inches, average fall being about 150 inches a year.

2. Total nitrogen brought down by rain is 8·290 lbs. per acre, considerably higher than that at Dehra Dun or Cawnpore and also at Rothamsted.

3. The ratio of ammonia to nitric nitrogen is as 1 : 0·82. The percentage is thus higher than in non-tropical countries—which is in accord with the current theory.

4. The biochemical activity is perhaps the most predominating cause regarding the source of ammonia at Sylhet.

5. The amount of albuminoid ammonia is 5·405 lbs. per acre.

In conclusion we wish to express our thanks to Mr. D. E. Roberts, Principal of the College, for granting us all facilities in connection with the work and for his kind interest in it.

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A NOTE ON THE INHERITANCE OF SEED-COAT COLOUR IN *PHASEOLUS LUNATUS* L.

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(Received for publication on 6th October 1932)

(With Plate XXIV)

INTRODUCTION.

There are numerous publications on the inheritance of seed-coat colour in *P. vulgaris* L. and other beans but the author has been unable to trace any publication dealing with inheritance of *P. lunatus*. Varieties of *P. lunatus* are extensively cultivated in the dry zone of Upper Burma and two varieties, Rangoon White Bean (*pebyugale*) and Rangoon Red Bean (*pegya*) are exported. The combined acreage under these two varieties in 1930-31 was 300,347. Since 1924 selection and breeding work has been in progress at Mandalay and as a side-line the following observations on the inheritance of seed-coat colour have been made. A white-seeded variety known as Moki Lima was introduced from Egypt in 1924 and this has been used as the white parent in the following crosses. The object originally in view was to combine some of the hardy characters of the small-seeded Burmese coloured types (*pegya*) with the large flat-seeded characters of Moki Lima. Unfortunately the F_2 generations were all badly damaged by cricket attack and an insufficient number of plants matured to give certain ratios but from the F_3 progeny it has been possible to gain some information about the inheritance of testa colour.

NATURAL CROSSING.

In 1928 plants of white Moki Lima and rose-speckled *pegya* were grown together and allowed to intertwine. In 1929 the white seed from the Moki Lima was planted separately and out of 556 plants 27 produced coloured seed, or 4·85 per cent. Moki Lima has a large flat somewhat triangular-shaped seed, whereas *pegya* is small and roundish. The Moki Lima shape is largely dominant over the *pegya* shape and so it was possible to ascertain that the reciprocal cross occurred more frequently. Out of 228 plants grown from the *pegya* seed which had been mixed with Moki Lima in the previous season, 42 plants developed seed approaching Moki Lima in shape, giving 18·42 per cent. This was considerably higher than expected. It is thought that the crossing is done by small insects. The high amount of

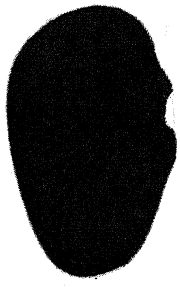
1.

2.

3.

4.

5.



6.

Fig. 1.—Purple dark speckled.
Fig. 2.—Purple light speckled.
Fig. 3.—Rose dark speckled.
Fig. 4.—Rose light speckled.
Fig. 5.—Purple plain.
Fig. 6.—Rose plain.

natural crossing has caused much trouble with this crop, which is not easily bagged. Artificial crosses, on the other hand, are difficult to perform successfully, any injury to the twisted keel resulting in abscission of the flower. Not more than 10 per cent. of successes have been obtained.

Moki Lima I × Pegya.

This cross was made in 1927, between Moki Lima I (white) and *pegya* (light rose-speckled on buff ground). The speckled character can be divided into two groups, dark-speckled and light-speckled (Plate XXIV, figs. 1-4). The degree to which the rose colour is suppressed is rather variable and it is not uncommon to find seeds in one pod which would singly be put in different classes, and also seeds which on one side are dark-speckled may occasionally approach the light-speckled group on the opposite side. In spite of these variations there is no difficulty in making the classification when several pods are examined from each plant. The F_1 was rose-speckled on buff ground but there was a much larger amount of rose than in the coloured parent, *i.e.*, it was dark rose-speckled. The F_2 (Plate XXIV, fig. 3) segregated 23 dark-speckled : 21 light-speckled : 10 plain rose : 9 white. In the F_3 generation the following segregations were observed :—

(*a*). *Dark rose-speckled on buff ground.*—None bred true. From four lines the following numbers were obtained :—

Dark rose	Light rose	Plain rose	White
22	9	14	15
56	21	32	40
159	75	64	86
199	100	100	108
436	205	210	249

Total=1100

Coloured	White
851	249
3·09	*0·91*

Wherever colour has occurred in the seed-coat and is broken up in an irregular manner (speckled) the ground colour is always a light buff. There are types of *P. lunatus* in which the ground colour may be white or pale green but they are not dealt with in this paper. In the above segregation there is clearly one colour factor concerned, **R**, which gives white in the recessive condition (**r**). If a gene **S** for speckling is assumed which in the homozygous state, **SS**, gives the light pattern and which when heterozygous, **Ss**, gives the dark-speckled class we obtain the following :—

—	Dark rose	Light rose	Plain rose	White
Observed	436	205	210	249
Calculated	412·50	206·25	206·25	275·00
(6 : 3 : 3 : 4)				

$\chi^2 = 4{\cdot}054$, P between ·3 and ·2.

The discrepancy, though rather large in the dark and white classes, does not invalidate the theory. It is to be noted that no darkly speckled rose beans have been found to breed true, which supports the above scheme.

(*b*). *Light rose-speckled on buff ground.*—This class either bred true or segregated into light-speckled and white. None produced either dark-speckled or plain rose. From seven lines the following numbers were obtained :—

Line No.	Light-speckled	White
291	192	75
292	293	88
294	58	17
296	135	25
298	29	5
2910	180	58
2911	55	16
	942	284

Ratio = 3·07 : 0·93.

In the light of the behaviour of the dark class it appears that the light class are homozygous for the speckling factor and can only segregate for the colour factor, **R.**

(*c*). *Plain rose* (*Plate XXIV, fig. 6*).—In this class the speckling does not appear and the buff ground colour which accompanies the speckling factor cannot be distinguished if it is present. Combining a number of lines the numbers obtained were 352 plain rose : 117 white = 3·002 : 0·998.

(*d*). *White.*—All bred true.

On the above assumptions the calculated F_2 would be,

Rose dark-speckled	Rose light-speckled	Rose plain	White
23	21	10	9 (Observed)
23·58	11·79	11·79	15·72 (Calculated, *6 : 3 : 3 : 4*).

The fit is far from good but in view of the small numbers and the behaviour of the F_3, it may be accepted as the best explanation available.

Moki Lima II × Pegya.

From a cross between another strain of Moki Lima and *pegya* purple colour was produced in the F_1 (Plate XXIV, figs. 1, 2 and 5). The speckling pattern with a buff ground developed in the same way as in the previous cross but the behaviour of the colour genes was different. In the F_2 the following segregation took place :—

Purple dark-speckled	Purple light-speckled	Purple plain	Rose dark-speckled	Rose light-speckled	Rose plain	White	Total
41	16	18	15	6	5	35	136

Considering first the colour characters,

Purple	Rose	White
41+16+18	15+6+5	35
=75	=26	35
8·82	3·06	4·11 (Theory, 9 : 3 : 4).

If a colour intensifier **P** is assumed which converts rose to purple so that **RP** gives purple but **P** is inactive when **R** is absent, the above numbers approximate closely to the modified dihybrid scheme indicated. The three genes, **R**, **P** and **S** would then lead to the following trihybrid scheme :—

Purple dark-speckled	Purple light-speckled	Purple plain	Rose dark-speckled	Rose light-speckled	Rose plain	White
18	9	9	6	3	3	16 (Ratio)
41	16	18	15	6	5	35 (Observed)
38·25	19·125	19·125	12·75	6·375	6·375	34 (Calculated)

X^2=1·1621, P=about ·97

Considering the small numbers the fit is good. We may therefore conclude that the constitution of the parents in this cross was

rrPPss × RRppSS

From the F_2 generation of this cross one plant gave the following progeny in F_3 :—

Purple dark-speckled	Purple light-speckled	Purple plain	Rose dark-speckled	Rose light-speckled	Rose plain
47	15	20	16	10	7
43·125	21·5625	21·5625	14·375	7·1875	7·1875 (Expected)

χ^2=3·7919, P=about 0·6.

Assuming the same factors as before and that the F_2 plant was homozygous for the rose factor, **R**, the theoretical ratio would be 6 : 3 : 3 : 2 : 1 : 1, and considering the small number the fit is about as good as could be expected.

Summary.

A gene, **R**, which produces a rose colour in the seed-coat of some varieties of *Phaseolus lunatus* L., is described and an intensifier, **P**, which converts rose into purple but which alone is inactive.

The speckled pattern is produced by the gene **S** which breaks up the rose or purple colour. It is a partial dominant, causing a large degree of colour suppression when homozygous and a much smaller amount when heterozygous.

A SIMPLE METHOD OF PREPARING CELLULOSE (HYDRATE) FOR CELLULOSE AGAR.

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Kellerman and McBeth [1912] developed a special culture medium for which cellulose was prepared in cuper-ammonium solution. The method as described by McBeth [1916] yields a satisfactory product, but the working of it involves a good deal of manipulation.

Scales [1915] suggested the following process: "100 c.c. of concentrated sulphuric acid are diluted with 60 c.c. of distilled water in a 2-liter flask and cooled to 60° or 65°C. To this are added 5 grams of moist filter paper, and the mixture vigorously shaken until filter paper is completely dissolved. The flask is then quickly filled with cold water and the precipitate filtered and washed until it is free from acid. When the volume of the suspension is drained to about 200 c.c., a hole is punched in the filter. The precipitate is then washed into a flask and the volume made up to 500 c.c."

The above method, when tried in this Laboratory, gave variable results. It was, therefore, considered necessary to so modify the Scales' process as to secure with ease cellulose hydrate of the desired quality.

One gram of filter paper was dissolved in 20 c.c. of concentrated * sulphuric acid diluted with 20, 18, 16, 14 and 12 c.c. of distilled water, and cellulose hydrate made to precipitate at 30°, 35°, 40°, 45°, 50°, 55°, 60°, 65°, 70° and 75°C., according to the Scales' method described above. Precipitates were made in triplicates. Each of them was washed acid-free, air-dried and weighed. The observations recorded in Table I indicate that the hydrate prepared in 20 c.c. of concentrated sulphuric acid diluted with 20 and 18 c.c. of distilled water at all temperatures was coarse and filamentous. 20 : 16 acid solution produced a coarse material at low temperatures, but it yielded a fairly satisfactory product between 50° and 60°C. At higher temperatures, the quality of cellulose hydrate was good, but bulk of the precipitate was markedly reduced. With 20 : 14 dilution the pre-

* Specific gravity of the concentrated sulphuric acid used was 1·841 at 60°F.

cipitate became flocculent and somewhat coarse at 30°C. but copious precipitate of fine quality was obtained at 40° to 45°C. At 50°C. the amount of precipitate was reduced considerably, until at and above 55°C. it became almost negligible. The precipitate obtained in 20 : 12 dilution at 30°C. was coarse, but at 35° to 40°C. it was fairly good. The amount of precipitate obtained at and above 50°C. was negligible for practical purposes.

The conclusion is, therefore, evident from the observations made above that the Scales' method yielded fairly fine cellulose hydrate with 20 : 12 and 20 : 14 dilutions at 35° to 40° C. and 40° to 45° C. respectively.

TABLE I.

Showing the influence of different concentrations of sulphuric acid and temperature on the precipitation of cellulose.

Acid : Water	10 : 9				10 : 8			
Temp.° C.	Nature of solution	Nature of precipitates	Amount of precipitates (air-dry)	Average amount of precipitates	Nature of solution	Nature of precipitates	Amount of precipitates (air-dry)	Average amount of precipitates
30 1	..	..	..	..	..	..	..	..
30 2	..	..	..	..	..	..	..	..
30 3	..	..	..	..	..	..	..	..
35 1	M	C & F	0·97	0·98	Solution	C	0·97	0·97
35 2	M	C & F	0·97		,,	C	0·98	
35 3	M	C & F	0·99		,,	C	0·99	
40 1	M	C & F	0·99	0·97	,,	C	0·95	0·97
40 2	M	C & F	0·95		,,	C	0·98	
40 3	M	C & F	0·97		,,	C	0·87	
45 1	M	C & F	0·97	0·95	,,	Fine	0·90	0·86
45 2	M	C & F	0·94		,,	,,	0·84	
45 3	M	C & F	0·96		,,	,,	0·85	
50 1	M	C & F	0·90	0·94	,,	,,	0·85	0·84
50 2	M	C & F	0·93		,,	,,	0·87	
50 3	M	C & F	0·98		,,	,,	0·80	
55 1	M	C & F	0·81	0·87	,,	,,	..	0·72
55 2	M	C & F	0·91		,,	,,	0·70	
55 3	M	C & F	0·90		,,	,,	0·75	
60 1	M	C & F	0·73	0·81	,,	,,	0·70	0·64
60 2	M	C & F	0·80		,,	,,	0·59	
60 3	M	C & F	0·90		,,	,,	0·45	
65 1	M	C & F	0·30	0·61	,,	,,	0·45	0·27
65 2	M	C & F	0·63		,,	,,	..	
65 3	M	C & F	0·91		,,	,,	0·10	
70 1	M	C & F	..	0·77	M	V ,,	0·1	0·04
70 2	M	C & F	0·71		M	,, ,,	Negl.	
70 3	M	C & F	0·84		M	,, ,,	0·11	
1	M	C & F	0·46	0·53	M	,, ,,	Negl.	0·03
2	M	C & F	0·56		M	,, ,,	..	
3	M	C & F	0·56		Turbid solution	,, ,,	0·10	

TABLE I—*contd.*

Showing the influence of different concentrations of sulphuric acid and temperature on the precipitation of cellulose—contd.

Acid : Water	10 : 7				10 : 6			
Temp.C.	Nature of solution	Nature of precipitates	Amount of precipitates (air-dry)	Average amount of precipitates	Nature of solution	Nature of precipitates	Amount of precipitates (air-dry)	Average amount of precipitates
30 1	Solution	Flocculent	1·04	1·02	Solution	Flocculent	0·97	0·96
30 2	,,	,,	1·01		,,	,,	0·90	
30 3	,,	,,	1·02		,,	,,	1·00	
35 1	,,	Fine	0·99	0·96	,,	Fine	0·95	0·83
35 2	,,	,,	0·95		,,	,,	0·73	
35 3	,,	,,	0·94		,,	,,	0·82	
40 1	,,	,,	0·94	0·86	,,	,,	0·55	0·49
40 2	,,	,,	0·98		,,	,,	0·41	
40 3	,,	,,	0·67		,,	,,	0·52	
45 1	,,	,,	0·95	0·86	,,	,,	0·34	0·15
45 2	,,	,,	0·86		,,	,,	0·10	
45 3	,,	,,	0·77		,,	,,	Negl.	
50 1	,,	,,	0·60	0·49	,,	V ,,	0·28	0·13
50 2	,,	,,	0·34		,,	,,	0·12	
50 3	,,	,,	0·55		,,	,,	Negl.	
55 1	,,	,,	0·13	0·16	,,	,,	,,	Negl.
55 2	,,	,,	0·10		,,	,,	,,	
55 3	,,	,,	0·27		,,	,,	,,	
60 1	,,	V ,,	0·10	0·03	,,	,,	,,	,,
60 2	,,	,,	Negl.		,,	,,	,,	,,
60 3	,,	,,	,,		,,	,,	,,	,,
65 1	,,	,,	,,	0·03	,,	,,	,,	,,
65 2	,,	,,	,,		,,	,,	,,	,,
65 3	,,	,,	0·11		,,	,,	,,	,,
70 1	,,	,,	Negl.	..	,,	,,	,,	,,
70 2	,,	,,	,,		,,	,,	,,	,,
70 3	,,	,,	,,		,,	,,	,,	,,
1	,,	,,	,,	..	,,	,,	,,	,,
2	,,	,,	,,		,,	,,	,,	,,
3	,,	,,	,,		,,	,,	,,	,,

M=Maceration C=Coarse F=Filamentous V=Very

A NEW METHOD OF PREPARING CELLULOSE HYDRATE

While working on the Scales' method a simpler process was developed for the preparation of cellulose hydrate of the desired quality. Filter papers were placed side by side in 50 c. c. of 10 per cent. sulphuric acid solution in a porcelain dish in such a manner that the lower portions of each were dipped in the acid solution and the upper portions left exposed to the air. The dish with its contents was then placed at 37°C. in the incubator. After 24 hours the position of the filter papers was gently reversed and the acid solution was drained off at the end of another interval of twenty-four hours. The filter papers were again incubated for a period of 48 to 72 hours when they had lost strength, breaking down even at a gentle touch. These were then carefully transferred over to the filtering apparatus and washed acid-free. When free from acid the material was thoroughly shaken with distilled water in a stoppered cylinder and fine suspension of cellulose hydrate was stored in the cooler for future use. The same product may be partly dried, ground in alcohol and made into a fine powder.

It was remarkable indeed that the particles of cellulose hydrate in water suspension made by this method were so fine that they did not completely settle down for days together, whereas the product made by the Scales' modified method settled down within 2-3 hours after precipitation. The extreme fineness of cellulose hydrate thus prepared gives this process a distinct advantage over the older method as it will pour the plates evenly. Besides, the method is very simple, requiring less sulphuric acid, no ice and affording greater ease in manipulation. It, however, takes longer to prepare cellulose hydrate by this method than by the modified method of Scales.

CONCLUSIONS.

1. It was found that cellulose dydrate precipitated but sparingly in 20 : 12 acid solution at 60°-65°C. as originally recommended by Scales.

2. Very satisfactory product was, however, secured by precipitating cellulose in 20 : 12 and 20 : 14 acid solutions at 35°-40°C. and 40° to 45°C. respectively.

3. The product made by the new method developed in this Laboratory, was found to be quite superior in quality.

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NOTES ON THE BIONOMICS OF *ODONTOMYIA CYANEA* BRUNETTI (DIPTERA: STRATIOMYIDAE).

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(With Plates XXV and XXVI)

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I. Introduction.

This work was started at the suggestion of Mr. P. V. Isaac, Second Imperial Entomologist (Dipterist) and was carried out while I was an Assistant in the Entomological Section of the Agricultural Research Institute, Pusa. I am indebted to Messrs. Isaac and M. Afzal Husain (who was officiating as Imperial Entomologist, in 1925-26) for their keen interest in this investigation and to Mr. T. B. Fletcher, Imperial Entomologist, for allowing me to remove the study material and the drawings on my transfer to the Forest Research Institute, Dehra Dun. I am grateful to artists, Messrs. P. Narayanan and V. G. Lele of Pusa for preparing the plates.

II. Technique.

The larvæ were killed in boiling water and their calcareous matter was removed by dilute hydrochloric acid. For the study of mouth-parts, the head was macerated in 10 per cent. potassium hydroxide, washed in glacial acetic acid, dehydrated, stained in picric acid, dissolved in clove oil and mounted in balsam.

III. Systematic position.

Several species of Odontomyia occur in India, and of these *Odontomyia cyanea* Brunetti, is very common at Pusa (Bihar) during the month of March. This species was first named as *O. violacea* in 1917 by Brunetti, but later [1920] he changed the name to *O. cyanea*.

The chief points of interest in the sub-family—Stratiomyinæ, to which this insect belongs, are, that the elongate antennæ are three-jointed, the third joint annulated and devoid of arista and that the posterior cross-vein is present. Brauer in 1883 describes the larvæ as peripneustic [Verrall, 1909]. Miall [1895] says there are, in larvæ of Stratiomyia, nine pairs of spiracles on the sides of the body, which are not open, though branches from the longitudinal air-tubes pass to them. On the other hand, Lundbeck [1907] mentions that there always are terminal spiracles lying in an oval fissure on the last segment fringed with feathery hairs. Thus with regard to the function of the tracheal system the larvæ could be termed metapneustic. The pupæ are enclosed in the last larval skin, differing in this respect from other Brachycera.

Miall [1895] states that the systematists consider the structure of the antennæ as an important character. The antennæ of Stratiomyia which is allied to Odontomyia, are considered as intermediate between the many jointed antennæ of Nematocera and that of Muscidæ. The intermediate position also reflects on the mode of its metamorphosis. It resembles Nematocera in structure and life-history except that it forms a more or less perfect, independent pupa-obtecta enclosed in the last larval skin, which it uses as a covering case. This condition also differs from the coarctate pupa of Cyclorrhaphous flies, although the two are superficially similar. The Muscid fly escapes by detaching a transverse fissure at the anterior segments; but the mode of emergence of the fly Stratiomyia is after the Nematoceran manner, that is, by splitting longitudinally, though there is also a transverse fission. It shows, then, that Stratiomyia and Odontomyia are the exceptions and important links between the Nematocera and Muscidæ, in possessing a more or less perfect pupa-obtecta formed within the last larval skin, thus indicating the origin of a change in the life-history.

IV. Habits and habitat of the larva.

The larvæ are found either floating in water accumulated in the hollows of trees or crawling in the mud and decaying vegetable matter collected there. A few larvæ were also found in the mud near the edge of a dirty drain at Pusa. These larvæ were noticed to live without water in a more or less desiccated condition for over two months and were also found to remain unaffected when kept in very

dilute salt solution; similar habits of Stratiomyia larvæ were observed by Malloch [1917] and Williston [1908] respectively. The Stratiomyid larvæ were found by John C. Hamm, in the thermal springs of Ninte, Wyoming [Johnson, 1895].

The larvæ come to the surface of water at times, and push their posterior extremities which carry the spiracles, into the air. The terminal long plumose hairs when spread out enable the larvæ to keep themselves at the surface and prevent the water from entering the respiratory chamber, and by their means when folded, they can retain a small bubble of air and carry it with them beneath the surface. The pointed tip of its tail-fringe pierces the surface film when the larva wants to come up; the feathery hairs set apart once more thus restoring an unwettable floating basin, which admits the air to the spiracle. The surface film is in a state of tension, *i.e.*, it exerts a pull, the advantage of which is taken by the larva by maintaining a vertical position, thus allowing the head and jaws to sweep through the water in search of food. It swims about with a vertical undulatory motion. It drags itself along by its mouth when out of water and alternate contraction and extension of the segments also help in forward movements. In shallow water, the last two or three segments are usually bent upwards, so as to reach the surface.

The larvæ have been noticed to feed upon other small insect larvæ and upon various decaying vegetable matter, swept into the mouth by the palps. Some have been found preying upon their own kind, which is probably in the absence of the usual food material. They were noticed to feed in congregation on dead pyralid worms, cockroaches and rotten fruits, when such type of diet was offered to them.

V. Life-history.

A number of larvæ were collected from the hollows of trees in the vicinity of Pusa Estate, in the last week of November, 1925. In February, 1926, larvæ reached maturity and had pupated and the adults began to emerge from the middle of March. Attempts to breed out a new generation in the laboratory were unsuccessful.

In 1926, material was again collected in the months of July and August. They remained in the larval stage throughout the rains and winter, and attained the fully-grown stage during February, 1927, and began to issue as adults in March and early April. Males and females were again caged for mating and oviposition but no eggs were laid. Special care was taken this time to offer, as far as possible, a natural surrounding for the flies to lay eggs. For this purpose the ground surface of the cage was covered over with a thick layer of odorous refuse brought from the hollows of trees and was kept wet throughout the experiment. Pieces of bark and leafy shade were also provided.

From the material collected, it appears that the larval period is very long and the insect remains in that stage throughout the summer and winter and becomes ready for pupation in the following spring.

At the time of moulting, the larva crawls out to the surface of refuse and lies inactive there. The outer skin starts drying and then (1) a slit occurs dorsally just behind the head and a transverse slit at right angles to it or (2) the outer skin becomes completely dried out and breaks up into pieces and thus exposes the new larva from inside.

Before pupation the larva stops feeding and becomes sluggish. Its body becomes a little contracted. The puparium, closely resembling the larva, is only at times aquatic, otherwise at the time of pupation the larva generally creeps out of the water. It either pupates by burying itself just beneath the surface of the soil or in the floating vegetation. After a quiescent stage the pupa is formed inside the larval skin or puparium.

The pupa is inactive and the pupal period has been observed to be about seven days. It was rather difficult to ascertain the exact pupal period because the pupa, though free, is formed within the last larval skin with no apparent change. The slight inflation of the anterior part of the puparium especially near the middle reveals the inside formation of the pupa. The maximum pupal period extends to eleven days under laboratory conditions. In some cases the emergence did not occur from the puparia due to either fungus attack or some bacterial disease which caused the inside pupa to rot.

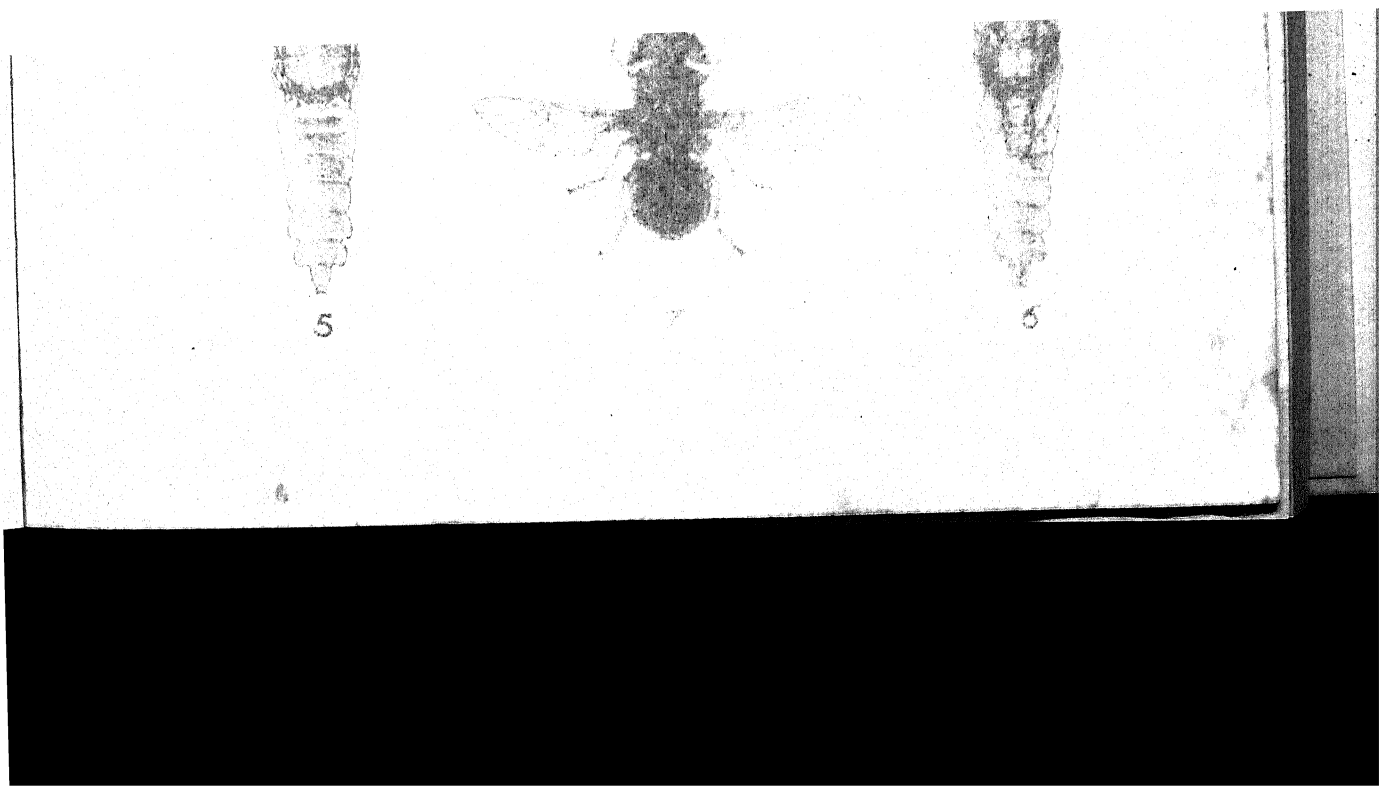
On the pupa becoming mature, the fly escapes, by splitting the skin transversely across the disc of the second segment, transversely on the fourth segment, and on the mid-dorsal line between these two, so as to form an I-shaped opening (Plate XXV, fig. 4). The adults commenced to appear from puparia from 5th March to 2nd April and 25th March to 18th April in 1926 and 1927 respectively. The adults fed in the cages on honey and water lived from 8-14 days.

Thus it appears that the life-cycle of this species is annual. The early spring brood of flies derived from the hibernating larvæ, gives rise to a summer brood of larvæ which remains in that stage throughout the winter, and which shows no emergence till the following spring.

V. Description of various stages.

(*a*) *Larva* (*Plate XXV, figs. 1, 2, 3 and Plate XXVI*).

From the collection made in 1925-26, the smallest larva measured was about 3 mm. in length and about 1 mm. in breadth and consisted of 12 blackish brown segments. Behind the small horny, slightly retractable head, which is covered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Odontomyia cyanea BRUNETTI.

1. Larva, dorsal view (× 2).

2. Larva, ventral view (× 2).

3. Last larval segment with a portion of penultimate segment, showing a pair of hooks, anal slit bounded by piliferous spots and feathery hairs, ventral view (× 6).

4. Empty puparium, showing emergence rent (× 2).

5. Male pupa, removed from puparium, dorsal view (× 3).

6. Female pupa, removed from puparium, ventral view (× 3).

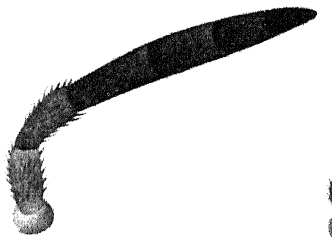
7. Male fly with expanded wings, dorsal view (× 3).

8. Female fly with expanded wings, dorsal view (× 3).

9. Antenna of female fly (× 30).

10. Antenna of male fly (× 30).

NOTE.—Figs. 1—4 show deficiency of black and dominancy of brown. The appendages in Figs. 5, 7, 8 should be black and in Fig. 6 brownish black.



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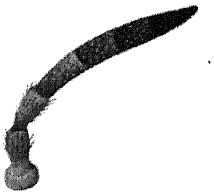

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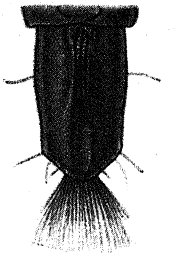

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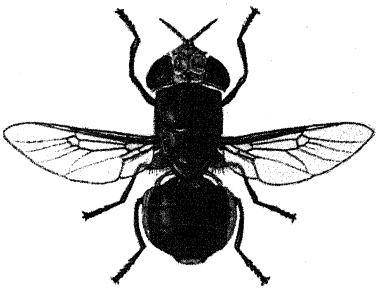

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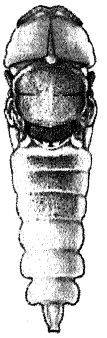

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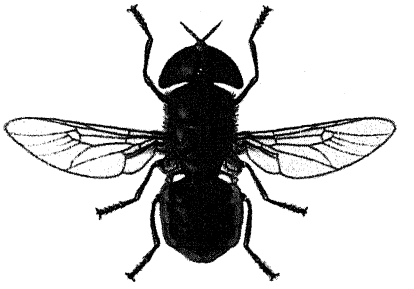

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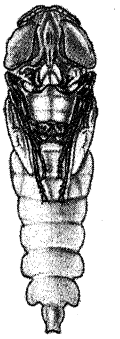

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PLATE XXVI.

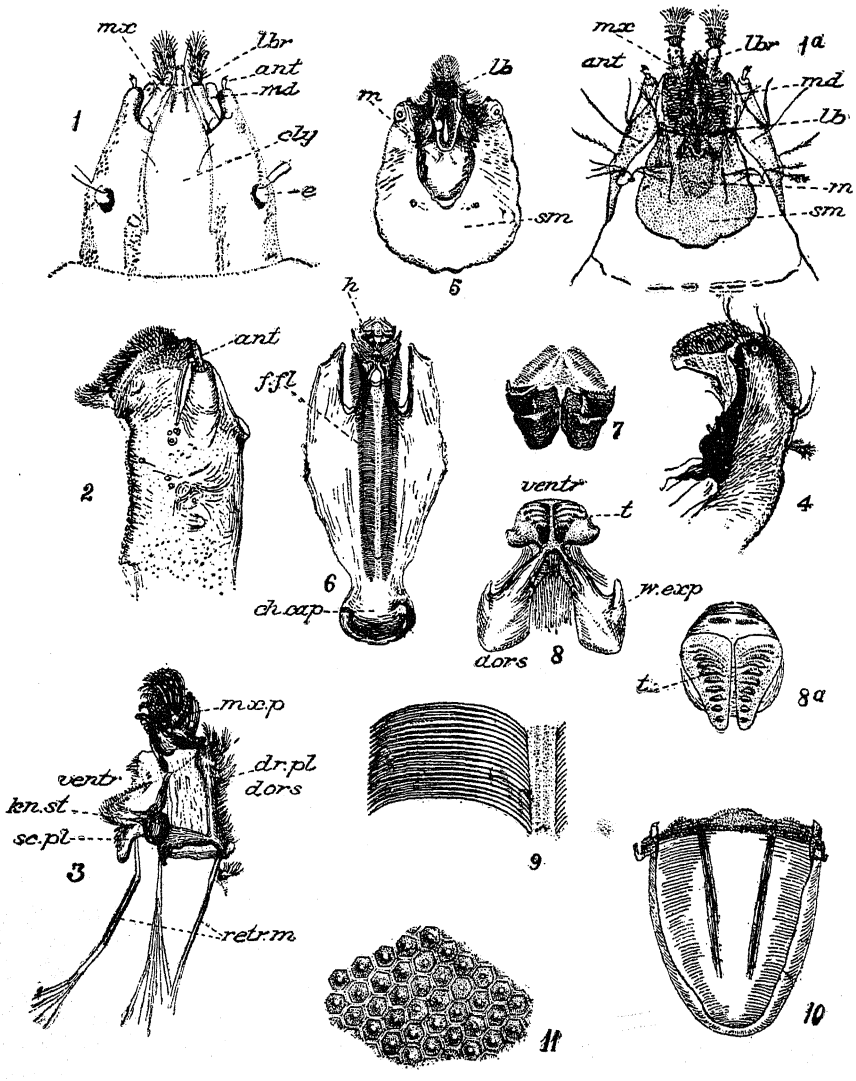
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PLATE XXVI.

Odontomyia cyanea BEUEN.

1. Head of larva, dorsal view (× 24). *lbr.* labrum, *ant.* antenna, *md.* mandible, *cly.* clypeus, *e.* eye, *mx.* maxilla.

1a. Head of larva, ventral view (× 24). (lettering as above). *lb.* labium, *m.* mentum, *sm.* submentum.

2. Mandible with lateral lobe (× 24).

3. Maxilla (× 24). *mx. p.* maxillary palp, *dr. pl.* dorsal plate, *kn. st.* knob-like structure, *sc. pl.* scale-like plate, *retr. m.* retractor muscles, *dors.* dorsal side, *ventr.* ventral side.

4. Labrum, lateral view (× 24).

5. Labium, mentum and submentum (× 26).

6. Tentorial skeleton (× 16). *h.* hypopharynx, *f. fl.* feathery flap, *ch. cap.* chitinised capsule.

7. Hypopharynx (× 34.

8. Rubbing structure (highly magnified). *t.* teeth, *w. exp.* winged expansion.

8a. Same, ventral view (highly magnified).

9. Portion of feathery lap (highly magnified).

10. Chitinised membran, ventral view.

11. Peltate scales.

by the front of the first segment of the body, come 11 segments which increase greatly in length and diminish in breadth towards the tail. The full-grown larvæ measured 25-30 mm. long and 4·5-5 mm. broad across the 4th and 5th segments which are widest (Plate XXV, figs. 1, 2.)

The first four segments are each slightly overlapped by the succeeding one, while the remaining segments are each slightly overlapped by the segment in front. The body is depressed and rigid due to the deposition of calcium carbonate by the hypodermis of the larva. The surface of the body is thickly covered with minute peltate scales (Plate XXVI, fig. 11) which help to stiffen it and also with few scattering hairs. It bears six light brownish longitudinal stripes on both sides of the body. On each side a black margin is visible along the entire length of the body. The stripes are all continued and are more distinct at the sutures but become faint along the margins of the segments and also in the last segment. The tough skin and colour probably protect them efficiently from insect enemies, in both larval and pupal stages.

There are nine pairs of spiracles which are not open, on the upper side of the lateral margins of segments first to tenth excepting on second. These spiracles are minute (except the first which is large), circular and brown in colour. Ventrally at the middle of the hind margin, on the fifth and on the tenth segment there is a pair of minute dark brown hooks, with their points curving towards the anterior (Plate XXV, figs. 2, 3). Hart [1895] describes the presence of one or more pairs of ventral hooks on the posterior margins of segments nine and ten, in his species of Odontomyia.

The sides of the last segment are almost parallel, tapering slightly towards the apex, with a terminal transverse cleft, which is furnished along its margin with long plumose hairs, about thirty in number. On the ventral side is an elongate anal slit, bounded by piliferous spots (Plate XXV, fig. 3).

The head (Plate XXVI, figs. 1, 1*a*) is small, sub-opaque and consists of a middle dark brown pointed lobe, which is separated by deep clefts from the lateral lobes. The palps bearing strong hairs are lodged in these clefts. The mouth is armed by a number of hooks; the eyes are small, dark, slightly prominent. A few plumose hairs are present on both the surfaces of the head.

The pointed median lobe forms a vertical, somewhat hooked labrum (a prolongation of the unnotched clypeus), which is bent ventrally and strongly sclerotised from the rest of the hind lobe (Plate XXVI, fig. 4). Anteriorly it is beset with hairs, some of which are conspicuously bristle-like on either side. Just behind these and situated dorsally are two bunches of hairs. To each side of the labrum lie the mandibles and maxillæ (Plate XXVI, figs. 1, 1*a*).

The lateral lobes bear at the point a small antenna (Plate XXVI, figs. 1, 1*a*, 2), which is composed of a basal segment bearing three small blunt sensory papillæ on its truncate apex and a terminal one having the form of a bell-shaped organ as in other Dipterous larvæ. Lying below these lateral lobes are the mandibles (Plate XXVI, fig. 2), which are weak and scale-like. Ventrally each mandible is provided with a dentate chitinous structure, having eight large teeth, curved somewhat inwards towards the mouth. The outer base of this dentate structure is beset with a tuft of long, stout hairs. Dorsally, the basal portion of the mandibles joins with the lateral lobes bearing antennæ and ventrally with the sclerotic submentum plate.

Below the antennæ and at the sides of the labrum are lodged on a small cleft of the tentorial apparatus a pair of maxillæ (Plate XXVI, fig. 3). They are very complex in life, moving rapidly and alternately with an upward and downward motion; and are provided with powerful retractor muscles. The maxilla consists of two basal plates (homologous to cardo) which are united dorsally in a sclerotic expansion or dorsal plate bearing sclerotised hairs. Ventrally the basal plates are richly provided with hairs, combs and teeth. One end of the basal plate is strongly sclerotised to form a knob-like structure, bearing two tooth-like processes. The front plate (which corresponds to stipes) is articulated dorsally and bears anteriorly 4-6 rows of hairs one behind the other in a regular manner. Ventrally, it is provided with a row of teeth. Above the rows of hairs, is situated the one-jointed cylindrical maxillary palp. The maxilla also carries a small accessory scale-like plate bearing four teeth pointing inwardly towards the mouth. This plate is considered by de Meijere [1917] to be an overgrowth of the maxilla and perhaps serves for chewing purposes [Becker, 1910]. This type of maxilla perhaps corresponds to the maxilla of the Eucephalen [Becker, 1910]. They serve the function of either locomotion in water or feeding or both.

The labium (Plate XXVI, figs. 1*a*, 5) which is a dark, small sclerotic piece, is situated ventrally and beset with fringe of hairs along its free edge. The submentum is a sclerotic plate carrying two pairs of plumose hairs and occupies the greater part of the ventral surface. It separates itself by its dark colouration from the lighter edges of the lateral lobes. Near the edges of the lateral lobes lie a pair of plumose hairs. The mentum is a small oval plate, the edges of which are strongly sclerotised. Just above the labium, lies the hypopharynx (Plate XXVI, figs. 6, 7) which consists of two small chitinous comb-like plates.

The tentorial skeleton consists of two longitudinal plates, the anterior lower margins of which are joined together medianally through a bridge, but towards the interior they become free from one another, thus forming an opening along its length in the median plane. This opening contains a pair of feathery flap-like

structures (Plate XXVI, figs. 6, 9). From the bridge commences the pharynx as a pressed tube. The side edges of the longitudinal plates are connected with the dorsal wall of the head which become independent internally and lie on either side of the pharynx as supports. The hind portion of the skeleton forms a sclerotised capsule which extends to the first thoracic segment, and contains a small, compact, spherical rubbing structure (Plate XXVI, figs. 8, 8*a*) bearing strong teeth ventrally. On the dorsal side it is provided with two winged expansions. Vaney considers this structure as the chewing apparatus [Becker, 1910].

The internal skeleton of the head capsule consists of a thin dorsal sclerotic membrane (Plate XXVI, fig. 10) or head plate (according to Jusbaschjanz terminology) and is a direct advancement of the dorsal head wall [de Meijere, 1916] which takes the origin internally from the anterior margin of the first thoracic segment and descends backwards up to the hind sclerotised capsular portion of the tentorial apparatus. The free margins of this membrane are bent inwardly and ventrally, thus enclosing most of the pharynx.

(*b*) *Pupa* (*Plate XXV, figs. 5, 6*).

The pupa is about 15 mm. long and about 4 mm. wide. It is creamy white in colour when newly transformed but becomes light yellow brown when about two days old. The appendages also assume a darker colour. The antennæ lie anteriorly along the margin of the eyes. The legs are glued down to the body on the ventral side and so are the wing-pads at the sides. The pupa of the male is a little smaller than that of the female and the distinctive sexual characters are obvious from the contiguous nature of the eyes in the male.

(*c*) *Adult* (*Plate XXV, figs. 7, 10*).

The adult is quite a large fly having deep metallic blue colour that gives it the appearance of a *Lucilia*. Brunetti [1923] states, "apparently the only oriental metallic species except *O. luteiceps* de Meij. which, however, has a black, non-metallic thorax". The sexes may be distinguished by the distance apart of the eyes which are holoptic in the male. The first and second antennal joints are nearly sub-equal and the third joint bears five annulations with a small apical style. The abdomen is broad and greatly flattened; the wings do not cover the sides of the body but lie parallel upon each other over the abdomen, and project beyond it. The scutellum bears two spines which stand out towards the abdomen.

The adults are frequently found on flowers and also sitting on leaves near tree-holes in shady places. They do not seem to possess any marked powers of flight and do not easily fly away when disturbed. When enclosed in cages they appear to be positively heliotropic.

VII. Parasites.

In the skin of a larva small rounded holes were noticed which were due to the escape of chalcids. The larva was dissected out, and 9 chalcid pupæ were extracted which unfortunately never transformed into adult forms.

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ORIGINAL ARTICLES

INHERITANCE OF FLOWERING DURATION IN RICE (*ORYZA SATIVA* L.).

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(With Plate XXVII and eleven text-figures).

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I. Introduction.

Rice in South India is principally transplanted. The seeds are first sown in a seed bed and after the lapse of some time, which depends upon the age of the varieties, the seedlings are transplanted in open fields with definite spacings which again depend on (*a*) the conditions of the seedlings at transplant time, (*b*) the age of the variety, and (*c*) the fertility of the transplant field. Review of relevant literature reveals some confusion in nomenclature, and in the pages that follow, it has been deemed necessary to adopt certain definitions to clarify the issue. Since the period between the emergence of the panicle and until it is ripe for harvest is a fairly constant one for all varieties, the duration of the crop for comparison purposes is best expressed in terms of flowering or panicle emergence. Thus

flowering duration is the total number of days that elapse between sowing of the seed and the beginning of the flowering phase. This definition holds good both with a broadcast and transplanted crop. *Time of flowering* is the date (*i.e.*, day of the month) on which flowering is first observed in the individual plant. A plant may have several tillers, each one of them producing a panicle, and as they cannot all flower on the same day, the time of flowering for the plant is taken as the day on which the first panicle emerges out of its leaf-sheath. In families where the time of flowering is specially sought to be studied, the plants are planted with regular spacings and the flowering date of each plant is marked on a specially prepared squared paper wherein each plant has a square. Every second day the whole block is traversed, plant by plant, and the dates of flowering of each plant entered on the squared paper. When the whole family finishes flowering, the figures in the squared paper are added up, tabulated and the necessary statistics calculated. Where the time of flowering for individual plants in a family is not so marked, the time of flowering of the family is taken as the day on which over 50 per cent. of the plants had put forth ears. This is arrived at purely from eye judgment, and with experience one can make a fairly reliable estimate. The interval between the beginning and ending of the flowering phase in a family is taken as the *period of flowering*.

Flowering duration is an important economic character since it is a definite proportion of the total life-period of a crop and this should have a bearing on the final yields. Physiologically a variety which has a short life-period cannot produce as much grain as another with a definitely longer life-period though exceptions to this general finding are not rare. In the breeding work at Coimbatore, it is usual to note the time of flowering for every unit that is grown on the station. In the present paper an attempt has been made to collect together all the relevant records in connection with the inheritance of flowering duration accumulated in the station over a series of years, and to give a Mendelian interpretation to the results recorded.

II. Conditions affecting the character.

The flowering duration is influenced by several environmental conditions, the chief of them being the time of sowing the seeds. Each important rice tract has got a definite rice season and there is a certain well-defined period when the rices are sown. Irrespective of small changes in the time of sowing the crop, the time of flowering is fairly constant, the flowering duration alone being affected by the time of sowing. Sowings done earlier to or later than the optimum time result in either increasing or decreasing flowering duration.

There is a very wide range in the flowering duration of rice varieties. In the pure line collections at the Paddy Breeding Station, Coimbatore, there are varieties where the flowering duration is as short as 60 days, while in others it is as much as 180 days with a great many intermediate times between these two extremes. To illustrate the effect of the time of sowing on the flowering duration, Table I gives the flowering duration of one of the pure lines, No. 24, sown on different dates in the year during two seasons. It is seen that with earlier sowings the flowering duration is longer and this gets less and less as the sowings are done later. For a change in the time of sowing to as much as four months in 1925-26, differences in the flowering duration are all within a week. The same result is borne out for the year 1927-28 where, however, owing perhaps to the prevailing weather conditions the flowering duration of November sowings has been delayed. In both the years, sowings done later than December have delayed flowering very considerably. It follows therefore that within certain limits, every variety has a minimum flowering duration, and very early or very late sowings extend the flowering duration considerably. This does not, however, hold good with *kar* or short duration varieties which, irrespective of the time of the year in which they are grown, do not change their duration materially.

TABLE I.

Flowering duration of pure line, No. 24, sown on different days with interval of a month.

Sowing date	Flowering duration in days	
	1925-26	1927-28
June 1st	138	..
July 1st	118	113
August 1st	98	98
September 1st	90	85
October 1st	94	88
November 1st	91	116
December 1st	95	98
January 1st	104	130
February 1st	224	231

Besides the time of sowing, other environmental conditions that affect the flowering duration are the spacing given to individual plants in the field, and the conditions of fertility of the plot. Giving the plants too wide a spacing, say more than 9 in. to 12 in., delays flowering by a few days. So also, there have been instances where the same variety, sown and transplanted in the same day but in

different fields, has flowered on different dates, the maximum difference observed being about 3 to 5 days.

III. Discussion of literature.

Several investigators have studied the inheritance of flowering duration on different cereals from the very early days of breeding work. Biffen [1905] noted that earliness was a simple dominant over lateness in wheat. Freeman [1919] in his wheat crosses interpreted duration on a multiple factor hypothesis. Caporn [1918] working in oats found earliness to be a function of three factors. Emerson and East [1913], in their studies on maize, found that a cross between an early and a late variety of corn produced an F_1 generation strictly intermediate between the parents in earliness. The F_2 more than filled in the gap between the parents in all cases and in one case had a range far below the mean of the early parent to above the mean of the late parent. The above references clearly indicate that the flowering time may both be simple and complicated. While the simple cases were interpreted on unit Mendelian factors, the complicated cases have been explained under the multiple factor hypothesis.

There have been some results published even regarding the inheritance of flowering duration in rice. Hoshino [1915] found that the flowering duration of F_1 was intermediate between the two parents but inclined more towards the early parent. In F_2 the range varied within the combined ranges of the two parents having the minimum frequency class in the middle. He interpreted the results of his F_2s and F_3s with the assumption of 2 Mendelian factors. In some of the cases which he could not explain with the help of the 2 factors, a 3-factor hypothesis satisfactorily explained the actual results. He also stated that he did not find a single case of transgressive inheritance. Van der Stok [1910] has investigated time of ripening. According to him, in the cross between early and late ripening varieties earliness is entirely or almost entirely dominant to lateness in F_1, although in one case its behaviour was quite reversed. Bhide [1926] found lateness to be a simple dominant to earliness in certain crosses of his, the ratio of 'lates' to 'earlies' in the F_2 being 2·8 : 1. But in certain others the dominance of lateness was not apparent and in such cases he assumed that the earliness or lateness was controlled by multiple Mendelian factors. Nomura and Yamasaki [1925] formulated that the flowering time in rice was governed by three factors, each of which prolonged the time to a certain extent, one factor more than the other. In their crosses, the F_1s were later than the late parent, and the F_2s gave 3 lates to 1 early. He stated that the different combinations of the three factors brought about different shooting times. Jones [1928] reported in his two sets of crosses the

occurrence of transgressive inheritance in the F_2s revealing the existence of more than a factor difference though apparently 3 : 1 ratio of earliness to lates could be obtained by grouping. The studies of F_3s and F_4s, however, definitely proved the existence of more than 2-factor difference.

IV. Material and methods of study.

The several crosses in which the study of the inheritance of flowering duration has been studied are all dealt with in the following pages. To overcome the difficulty of environmental influences, in crosses where flowering duration was under study, the parents were sown and transplanted by the side of the F_1 and F_2s every year, so that the flowering records would be comparable. It is generally found that in a family pure for the flowering duration, early, medium or late, the flowering frequencies when plotted give a normal curve, the variation between the earliest and the latest being about a week to ten days. Where the family is not pure for the character, the frequencies either give multimodal curve or even a unimodal curve with a greater range in the time of flowering of the individual plants. Fig. 1 represents the flowering graphs of two single plant selections, 140 and 201. It is seen that the curves are normal, and that the range does not exceed ten days in both the families. Selections were made in each of the two families with flowering dates of a week's interval and another generation raised. Table II gives the details about these two families.

Table II.

Flowering data of two families apparently pure for flowering duration and the selections from them.

—	Family number	Flowering duration of parents (days)	Period of flowering (days)	Mean duration (days)	S. D.	C. V.
	140	..	128-136	131·9±·1	1·6	1·2
Selections from 140	657	128	132-152	140·9±·2	3·9	2·7
	658	128	136-150	141·3±·1	3·0	2·1
	659	136	136-150	142·2±·1	2·6	1·9
	660	136	140-154	144·2±·1	2·5	1·7
	201	..	138-147	141·5±·1	2·9	2·1
Selections from 201	661	138	151-163	155·8±·1	2·0	2·1
	662	138	149-163	155·5±·1	2·3	1·5
	663	145	149-161	153·7±·1	2·2	1·4
	664	145	151-163	156·2±·1	2·6	1·6

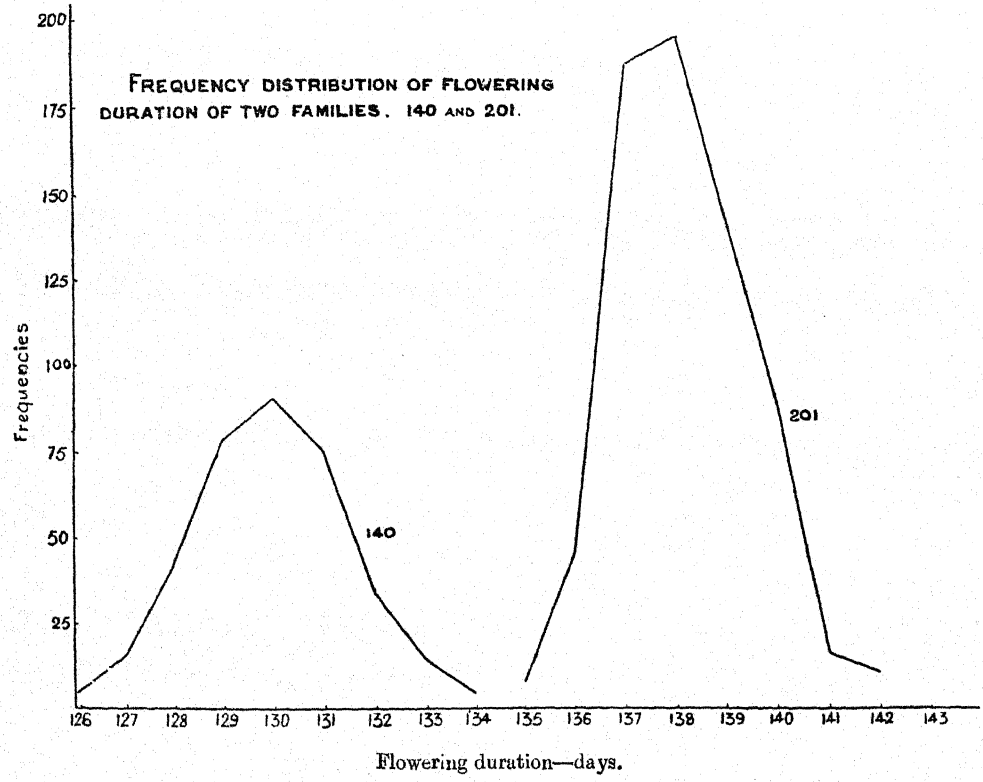


Fig. 1.

Although there was an interval of a week in the flowering time between the selection, there is no significant difference in the mean flowering durations of the progeny, and the standard deviations and co-efficients of variation are all very uniform. It should therefore be assumed that both the families are pure for flowering duration and the difference of 9 days obtained between the earliest and the latest plant was only due to the fluctuation of the character and not to any genetic impurity. To compare with the results of the above two families the behaviour of family No. 237 (Fig. 2) may be considered. The essential details of the flowering data of this family and the selections from the same are given in **Table III.**

TABLE III.

Flowering data of a family 237, apparently splitting for flowering duration.

—	Family number	Flowering duration of parents (days)	Period of flowering (days)	Mean duration (days)	S. D.	C. V.
Selections from 237	237	—	127-142	135·4±·1	2·8	2·0
	665	127	132-141	137·8±·1	2·6	1·9
	668	134	139-157	147·3±·2	3·7	2·5
	669	141	148-159	153·2±·1	2·0	1·3

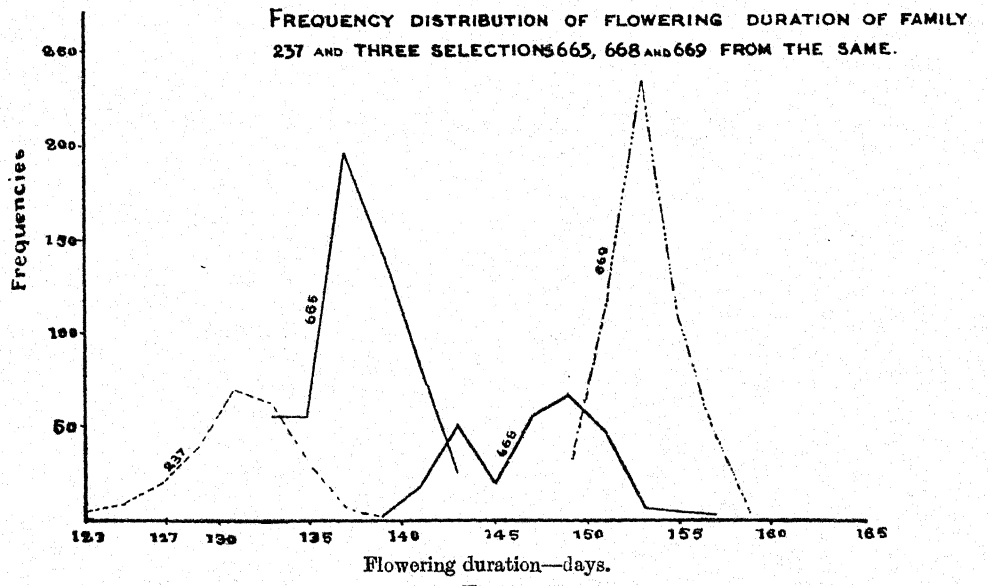


Fig. 2.

The flowering frequency of family 237 gives apparently a normal curve but with an extended range. Unlike families 140 and 201, selections taken with an interval of a week in flowering time give different ranges and different mean flowering durations. It would appear that family 237 has not been pure genetically for flowering duration. With this preliminary knowledge about the character, we can now proceed to examine the cases of definite crosses made on the station, wherein this character, flowering duration, was involved.

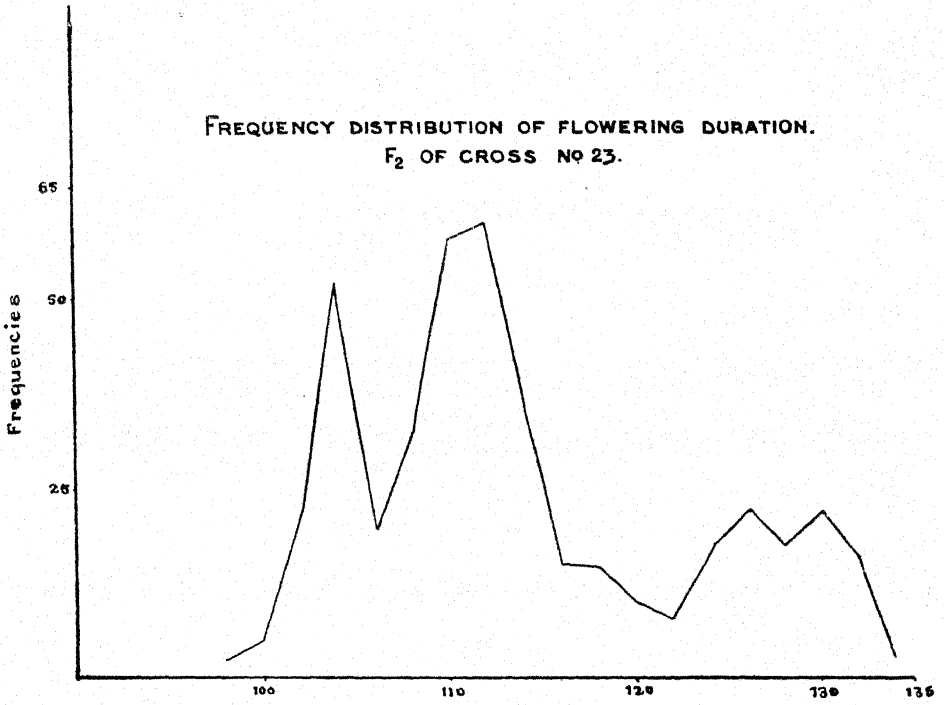
V. Analysis of cases—simple.

1. The first cross to be considered in connection with the study of flowering duration is No. 23. The flowering time of the F_1 had not been noted. The F_2 generation was studied individually for flowering time. Fig. 3 gives the F_2 distribution. It probably represents a trimodal curve with 1 : 2 : 1 ratio of early : intermediate : late, the actual ratios being 100 : 226 : 107 grouped according to the minimum frequency points. A number of selections were made in F_2 at different points in the curve and an F_3 raised therefrom.

Table IV.

Flowering data of the F_2s, F_3s and F_4s of cross No. 23.

Generation	Number of family	Flowering duration of parents (days)	Period of flowering (days)	Mean duration (days)	S. D.	C. V.
E. Parent	..	..	..	98	..	..
L. Parent	..	..	..	143	..	..
F_2	639	..	98-134	114·1±·4	9·0	7·9
F_3	1013	99	99-129	116·3±·2	3·8	3·6
	1005	103	103-125	113·7±·1	4·0	3·5
	1016	107	108-130	119·1±·1	3·5	2·9
	1003	113	110-154	129·0±·2	*8·3*	*6·4*
	1006	114	110-156	129·2±·3	*8·8*	*6·8*
	1007	122	119-145	134·5±·1	3·8	2·7
	1008	125	127-149	139·3±·1	3·1	2·2
	1011	129	125-153	142·5±·1	4·2	2·9
	1014	137	139-161	146·7±·2	3·4	2·4
From F_4 (1005)	1321	107	108-122	116·6±·1	2·9	2·5
	1322	109	102-120	113·5±·1	3·4	3·0
	1323	117	101-122	112·0±·1	3·5	3·2
	1324	119	104-123	113·1±·1	2·7	2·4
From F_4 (1016)	1351	116	108-132	118·0±·1	3·6	3·1
	1352	118	105-129	118·0±·1	3·2	2·7
	1353	122	107-129	118·4±·1	3·5	3·0
	1354	124	107-133	120·3±·1	3·4	2·8
From F_4 (1008)	1325	137	122-142	130·9±·1	2·8	2·1
	1326	139	124-144	133·1±·1	3·3	2·5
	1327	141	124-144	132·1±·1	2·8	2·1
	1328	143	126-150	135·9±·2	4·4	3·2
From F_4 (1010)	1333	135	118-136	125·4±·1	3·1	2·5
	1334	135	118-138	125·3±·1	2·6	2·0
	1335	139	122-138	130·8±·1	2·6	2·0
	1336	139	122-144	132·6±·1	2·9	2·2



Flowering duration—days.

Fig. 3.

Table IV gives the flowering data of the F_2s, F_3s and F_4s. When the flowering frequencies of the different selections from the F_2s were plotted on a graph paper, the curves obtained were all apparently normal showing no segregation except two families, 1003 and 1006, which were taken towards the middle of the F_2 graph. Fig. 4 gives the flowering frequencies of these two families. The figures apparently represent a bimodal curve giving a simple 3 : 1 of early to late. Taking the segregation at the minimum frequency class we get

	Early	Late	
Family 1003	1285	349	
Family 1006	618	225	
Total .	1903	574	Dev. / S. E. = 2·0
Cal. 3 : 1	*1858*	*619*	

Although in the F_2, the range of variation in the early group was from 99 to 127 days, selections with duration between 99 to 109 days were all apparently pure earlies in the F_3, because the mean duration of all these families varied only between 113 and 116 days. The two splitting families 1003 and 1006 gave a mean flowering duration of about 129 days. All the selections in the late group of the

F_2, *i.e.*, those with flowering durations beyond 123 days have all bred pure for lateness. The mean parental flowering duration of these late selections varied from 122 to 137 days and the mean durations of the F_3s vary from 135 to 146 days. This point was well borne out even in the F_4 behaviour.

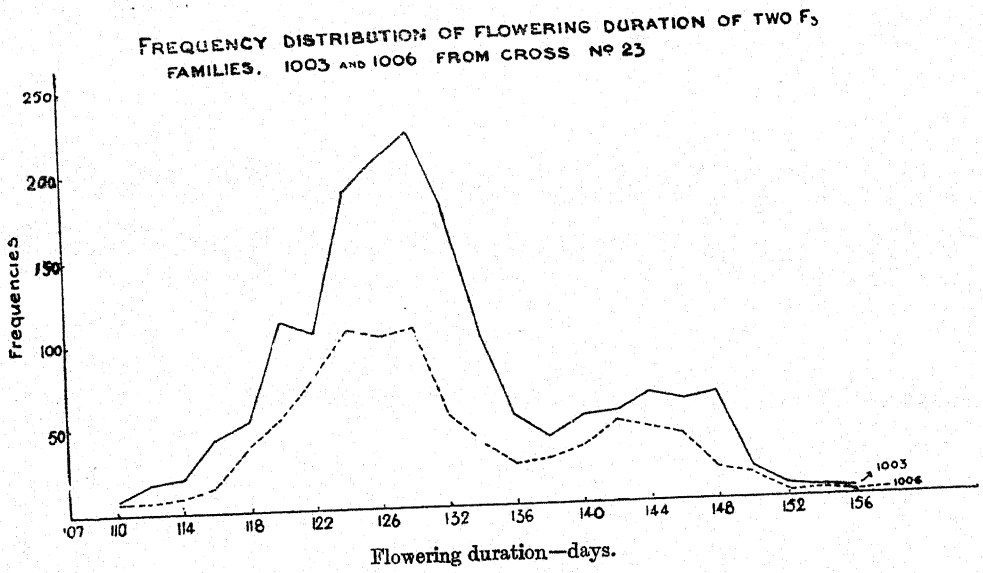


Fig. 4.

From the behaviour of this cross, it would appear that earliness and lateness behave as a simple pair of Mendelian allelomorphs. The extracted early type has a higher mean duration than the early parent and the extracted late type has a somewhat smaller mean duration than the late parent. There is thus definite evidence of *shift* for the character.

2. Another simple case of flowering duration inheritance comes from the study of a natural hybrid isolated from one of the pure lines, T. 100. In the plot of T. 100 just at flowering time, a plant was isolated as a possible hybrid and the F_2 was grown in the following season. The family was observed to be segregating for flowering duration, but no counts were taken. About 60 selections taken from F_2 and grown as F_3 showed, that the families were behaving differently for flowering duration. While some families were uniformly early, and certain others uniformly late, there were some where the flowering appeared to come off definitely in two flushes, earlies and lates ; 18 were pure earlies, 28 were giving both earlies and lates and 14 were pure lates.

The above figures represent a fair 1 : 2 : 1 ratio. A simple separation of the plants into earlies and lates was made in a few of the 28 segregating families and the results are given in Table V.

TABLE V.

Ratios of earlies to lates in F_3s and F_4s of a natural hybrid in T. 100.

Generation	Family number	Character of parent	Pure Earlies	Segregating into		Pure Lates
				Earlies	Lates	
F_3	2424	..	..	396	124	
	2427	..	..	450	175	
	2433	..	..	300	112	
	2434	..	..	517	180	
	2470	..	..	326	183	
	2477	..	..	648	185	
F_4 from 2427	2726	Early	Pure	..	..	
	2727	,,	,,	..	..	
	2732	,,	,,	..	..	
	2725	,,	..	751	258	
	2728	,,	..	301	121	
	2729	,,	..	229	116	
	2730	,,	..	464	135	
	2731	,,	..	405	157	
	2733	,,	..	416	170	
	2734	,,	..	437	207	
	2735	,,	..	834	118	
	2736	,,	..	784	172	
	2737	,,	..	773	289	
	2738	,,	..	690	168	
	2739	Late	..	..	..	Pure
	2740	,,	..	..	..	,,
	2741	,,	..	..	..	,,
	2742	,,	..	..	..	,,
F_4 from 2434	2744	Early	Pure	..	..	
	2746	,,	,,	..	..	
	2747	,,	,,	..	..	
	2750	,,	,,	..	..	
	2752	,,	,,	..	..	
	2745	,,	..	706	141	
	2748	,,	..	420	132	
	2749	,,	..	429	141	
	2751	,,	..	598	273	
	2753	,,	..	650	163	
	2754	,,	..	787	249	
	2755	,,	..	561	190	
	2756	Late	..	..	..	Pure
	2757	,,	..	..	..	,,
	2758	,,	..	..	..	,,
	2759	,,	...	..	..	,,
	2760	,,	..	..	..	,,
	2761	,,	..	..	..	,,
Total for the 24 splitting families .				12,872	4,159	Dev.
Calculated 3 : 1				*12,773*	*4,258*	$\frac{\text{Dev.}}{\text{S. E.}} = 1{\cdot}8$

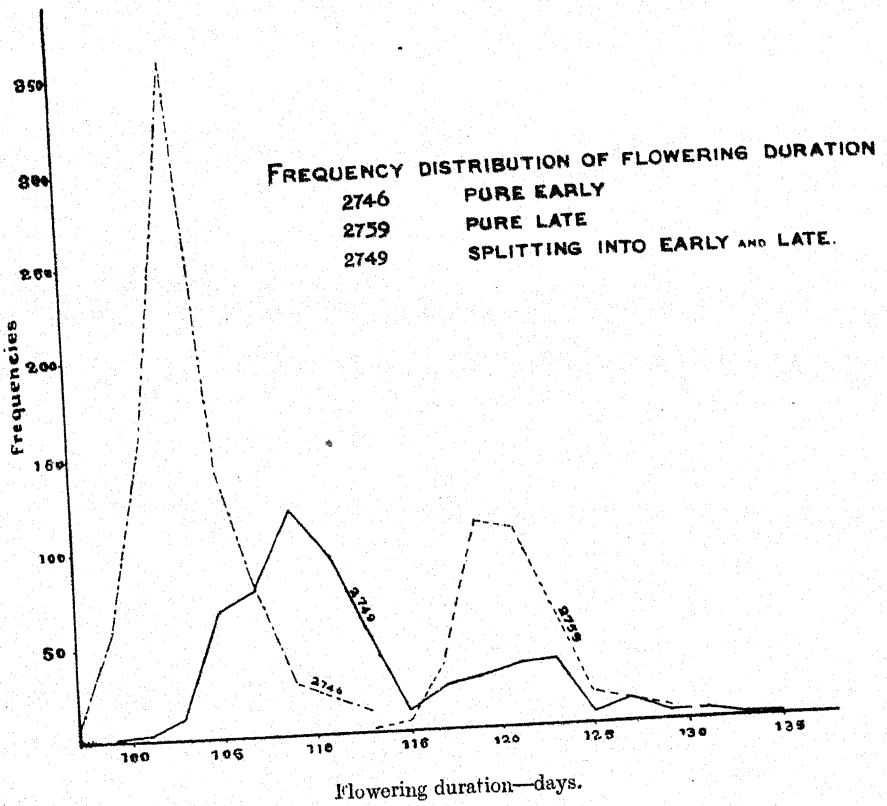


Fig. 5.

Fig. 5 represents graphically a typical example of one pure early family, one segregating family and one late family of the F_4s. This demonstrates once again the simple dominance of earliness over lateness. It was found that all the pure earlies had flowering ranges between 116 and 132 days with means varying from 121-128 days. The pure lates had flowering ranges from 130 to 150 days with means varying from 137 to 141 days. The ranges of all the segregating families covered the entire range of the two, pure early and pure lates. The mean flowering duration of one of the parents of the original hybrid was found to be between 116 and 120 days. The extracted pure earlies from F_4 roughly correspond to this though with a slightly higher flowering duration. The results can thus be interpreted with a single factor E_1 responsible for earliness and dominant to lateness e_1. The extracted early type does not strictly correspond to the early parent in its flowering duration, the same

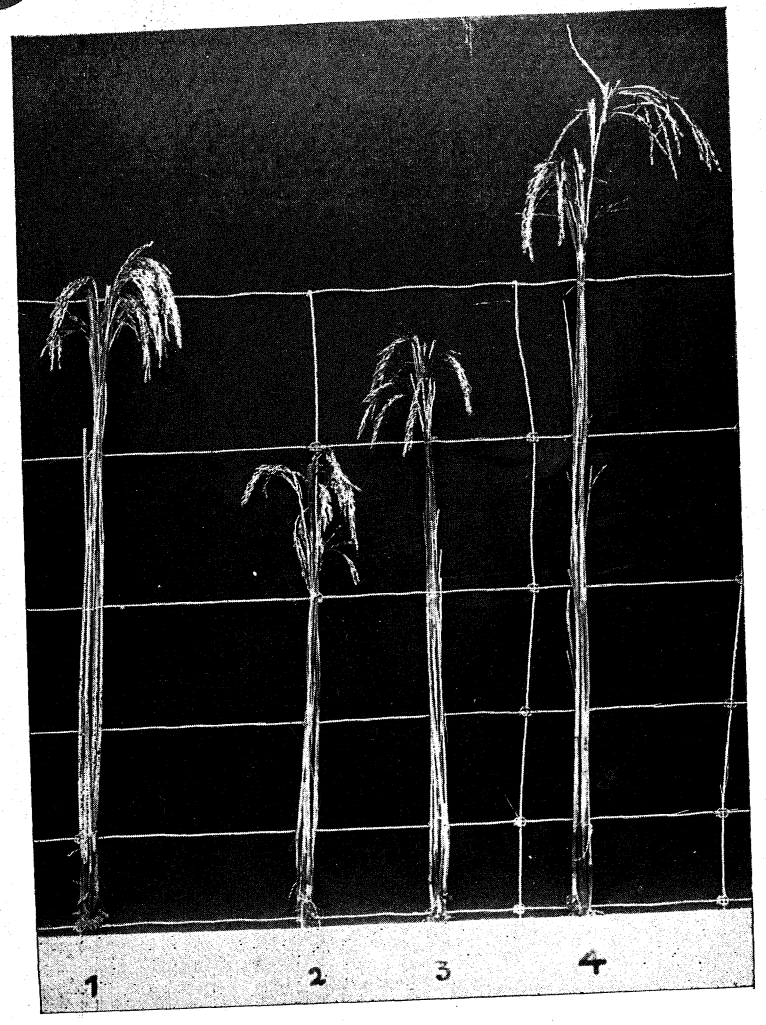


Fig. 1.

(*1*) T. 100, flowering duration 118 days. (*2*) and (*3*) Extracted early types, flowering duration 125 days. (*4*) Extracted late type, flowering duration 138 days.

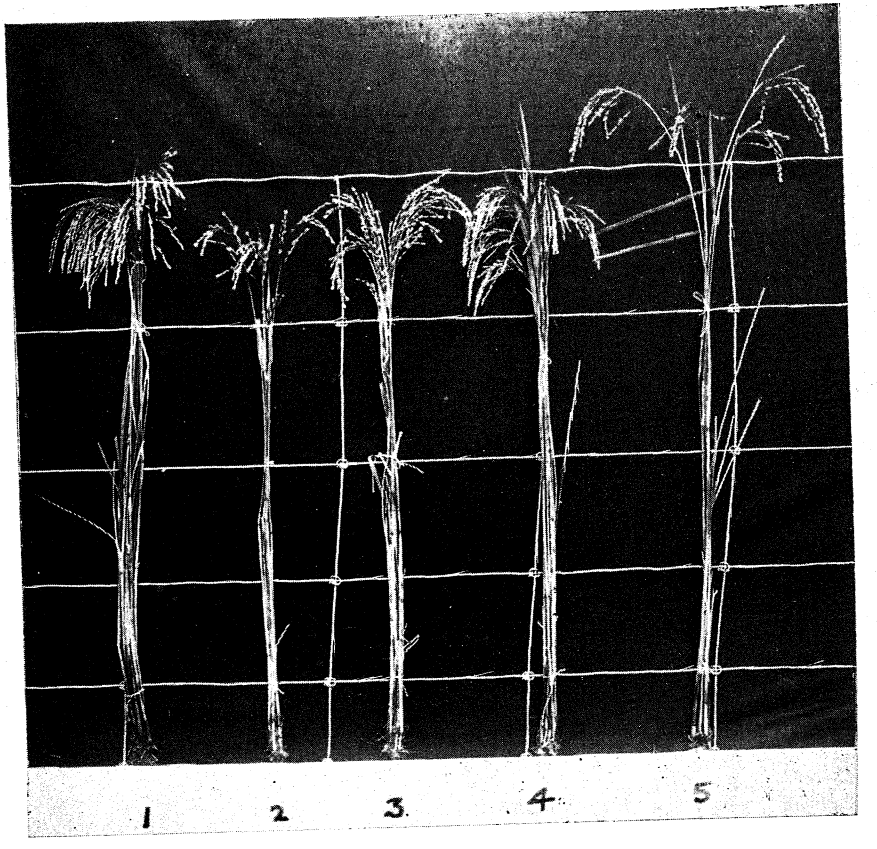


Fig. 2.

(*1*) T. 24, flowering duration 100 days. (*2*) T. 310, flowering duration 85 days. (*3*) and (*4*) Extracted types, flowering duration 99 days. (*5*) Extracted type, flowering duration 118 days.

as in the previous cross. Plate XXVII, fig. 1, depicts the extracted early and late types from this cross.

VI. Analysis of cases—complicated.

1. Cross T. 24×T. 310.

The parents T. 24 and T. 310 had flowering durations of 100 and 85 days respectively with a clear difference of a fortnight between the two. The flowering duration of the F_1 was 86 days almost like the early parent, T. 310, indicating complete dominance of earliness. The F_2 was studied individually for flowering time and the frequencies, when plotted on a graph paper, did not show any sort of breaks, and it was an apparently normal unimodal curve, indicating that the flowering duration was controlled by multiple factors. The flowering duration of the early parent was different in the 2nd year when it was being grown along with the F_2 due to the difference in the sowing time and season, but still the mean flowering duration of the F_2 was just like the F_1 in resembling the early parent.

	Period of flowering (days)	Mean flowering duration (days)
1st year—		
T. 24		100
T. 310		85
F_1		86
2nd year—		
T. 24	97-107	101
T. 310	85-97	91
F_2	81-107	92

The flowering ranges of the two parents just meet and do not overlap each other, and the F_2 has a range starting below the *minus* extreme of the early parent and just reaches the *plus* extreme of the late parent. A large number of selections made in the F_2 for the study of panicle character, could not be studied individually for flowering duration but the average time of flowering of the several individual families had, however, been recorded, which varied from 83 to 107 days. Of the selections made in some of the F_3 families and grown as F_4, two gave a sharp segregation into earlies and lates. For simple eye judgment there were two distinct flushes separable into 2 groups, earlies and lates. Since the segregation was sharp, simple counts were made of earlies and lates. These counts (Table VI) gave a simple 3 : 1 of earlies to lates, and the same behaviour was apparent in the selections grown as F_5s.

TABLE VI.

Ratios of Earlies to Lates in F_4s and F_5s of T. 24×T. 310.

	Family number	Character of parent	Pure early	Splitting into		Pure late	Remarks
				Early	Late		
F_4	5283			130	39		
	5284			121	49		
	5285			111	54		
F_5 Selections from 5283	5772	Early	Pure				
	5770	,,		76	28		
	5771	,,		77	27		
	5773	,,		150	57		
	5774	,,		74	30		(Only 2 rows of plants were planted in each family and hence the small number)
	5775	,,		71	33		
	5776	Late				Pure	
	5777	,,				,,	
F_5 Selections from 5284	5781	Early	Pure				
	5778	,,		84	20		
	5779	,,		79	25		
	5780	,,		73	31		
	5782	,,		72	32		
	5783	,,		72	32		
	5784	Late				Pure	
	5785	,,				,,	
Totals for 13 splitting families . . .				1,190	457		Dev. $\frac{}{\text{S. E.}}$ = 2·5
Calculated 3 : 1				*1,235*	*412*		

To make sure of the nature of segregation, 8 selections were made in two of these families and an F_5 was grown. The results are included in Table VI. The ratios between 'earlies' and 'lates' undoubtedly represent a monohybrid one. Pure breeding types have been extracted from these F_5s for earliness and lateness and those that have been grown on the station for three or four seasons give a clear difference of about 12-14 days in their flowering duration, irrespective of the season. The average flowering durations of the extracted early and late types are 99 and 118 days. While the early type corresponds to the late parent in duration, the extracted late type is much later in duration than the late parent.

The results can be explained with the help of two factors E_1 and E_2 controlling earliness. Each of the parents that went into the cross should be assumed to contain one of these factors. The absence of both the factors should make a type have

a flowering duration later than the late parent and the presence of both the factors should give a type earlier in duration than the early type.

T. 310 . . . . . $e_1e_1E_2E_2$ early parent.
T. 24 . . . . . $E_1E_1e_2e_2$ late parent.

The F_1 would be $E_1e_1E_2e_2$ giving the four types $E_1E_1E_2E_2$, $E_1E_1e_2e_2$, $e_1e_1E_2E_2$ and $e_1e_1e_2e_2$ in F_2. The two middle classes would correspond to the two parents, and of the two others one will be earlier than the early parent and the other will be later than the late parent. If the F_4 selections, 5283-85, are taken to have been of the constitution $E_1e_1e_2e_2$ these would give three $E_1E_1e_2e_2$ to one $e_1e_1e_2e_2$. Since $E_1E_1e_2e_2$ is the constitution of the late parent, the three would correspond to the duration of the late parent and the one $e_1e_1e_2e_2$ would be later than the late parent, which is exactly what happened. The factorial interpretation offered thus falls in line with the actual results obtained. Plate XXVII, fig. 2 shows the two parents and the extracted types from the F_4s.

2. Cross T. 24×T. 33.

The parents are both of very nearly the same duration as shown below. The F_1 was earlier than the two parents and in F_2 there was transgressive variation exceeding the parental limits on either side.

		Period of flowering (days)	Mean flowering duration (days)
1st year—			
	T. 24 parent		96
	T. 33 „		96
	F_1		89
2nd year—			
	T. 24 parent	96-102	99
	T. 33 „	98-104	101
	F_2	84-114	99

The F_2 flowering distribution approaches a normal curve (Fig. 6) and would seem to indicate that flowering duration is in this case controlled by multiple factors. The parents, though of the same duration, must have been different constitutionally for the factors controlling duration. The F_3 selections were not studied individually for flowering duration, but they exhibited a wide range in duration, 94-116 days. As a result of further work done in this group for quite a different purpose, pure types have been extracted with average durations exceeding those of the parents. This cross again illustrates quite clearly that flowering duration is controlled by multiple factors.

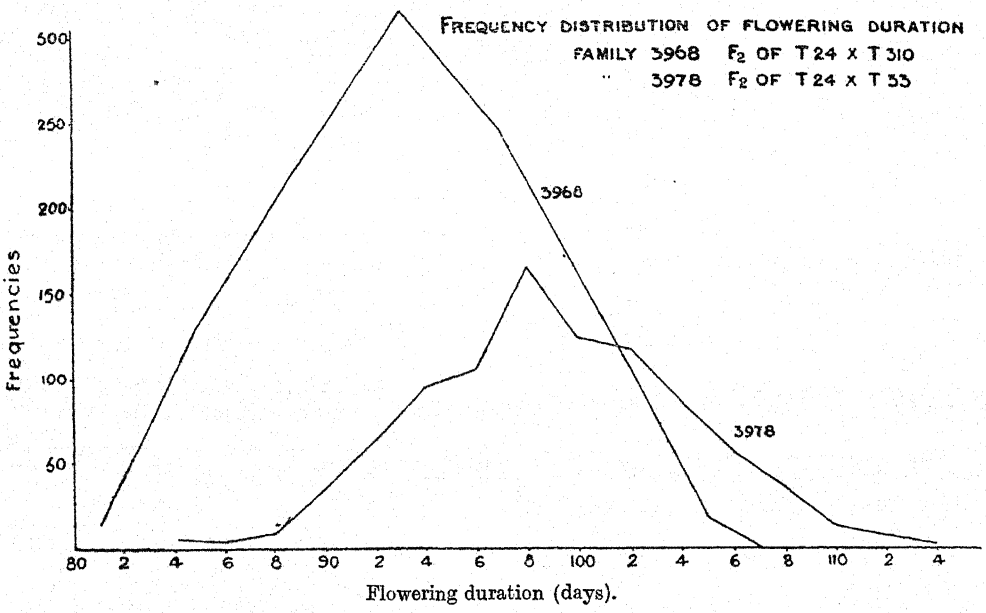


Fig. 6.

There have been several similar cases where the two parents have been nearly of the same durations, while the F_2s had given transgressive variations exceeding the parental limits on either side. As instances of these, a few are mentioned in Table VII.

TABLE VII.

Mean flowering duration and period of flowering of the parents with their F_1s and F_2s showing transgressive variation.

No.	Cross	MEAN DURATION (DAYS)			PERIOD OF FLOWERING (DAYS)		
		Parent	F_1	Parent	Parent	F_2	Parent
1	T. 47 × T. 103	110	96	105	102-118	84-128	100-108
	Mean duration of F_2	..	..	..	..	*103*	..
2	T. 47 × T. 103	105	96	104	102-114	82-126	102-108
	Mean duration of F_2	..	..	..	..	*101*	..
3	T. 185 × T. 1	118	112	112	113-125	97-141	107-119
	Mean duration of F_2	..	..	..	..	*113*	..
4	T. 1 × T. 92	113	112	107	108-120	90-130	102-116
	Mean duration of F_2	..	..	..	..	*110*	..
5	T. 97 × T. 87	114	107	105	108-122	90-128	98-114
	Mean duration of F_2	..	..	..	..	*104*	..
6	T. 1 × T. 1880	113	109	105	108-120	82-138	98-114
	Mean duration of F_2	..	..	..	..	*111*	..

In almost all these crosses, although the families were not studied individually for flowering duration beyond the F_2s, pure breeding types have been extracted from several of such crosses, some of which are of the same duration as the parents, while others are definitely earlier to or later than either parent. All these cases exhibit the characteristic of multiple factor inheritance.

3. Cross T. 24×T. 282.

T. 282 is a special variety obtained from Burma with a dwarf habit. The cross was intended to confirm the results previously obtained about the inheritance of the dwarf habit. The study of inheritance of flowering duration came in incidentally. The main results concerning this character are as follows :—

		Period of flowering (days)	Mean duration (days)
1st year	T.24 parent	..	96
	T.282 „	..	108
	F_1	..	100
2nd year	T.24 parent	98-102	100
	T.282 „	104-118	110
	3980 F_2	96-118	103

The flowering duration of the F_1 is not strictly intermediate but inclined more towards the early parent. The mean duration of the F_2 is also about intermediate slightly inclined towards the early parent.

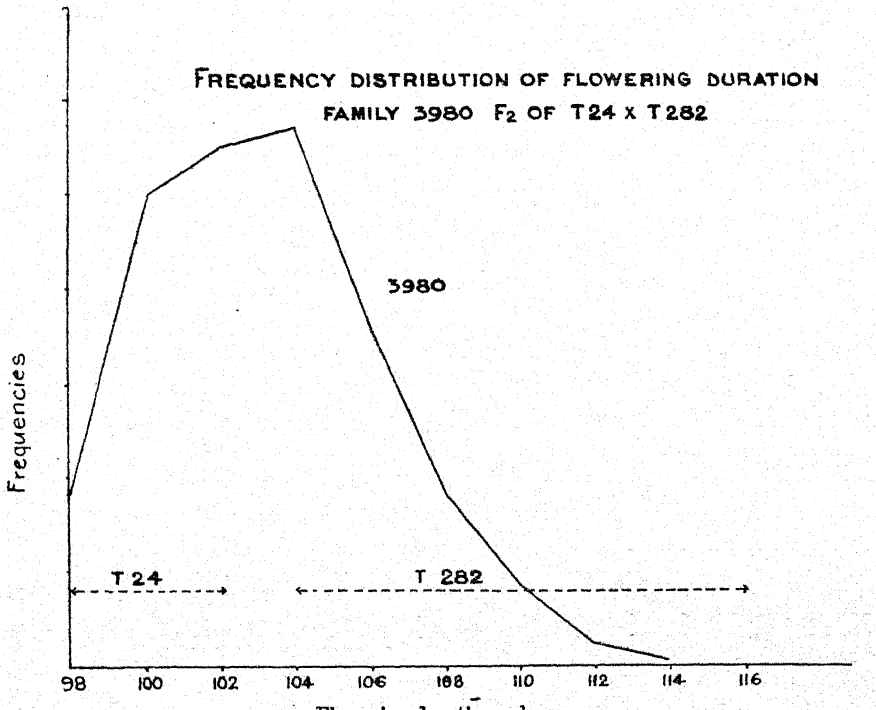


Fig. 7.

The F_2 frequency plotted in Fig. 7 is lop-sided towards the early parent and there is no indication of any break. The inheritance is not apparently simple. The large number of selections grown as F_3 were not studied for the flowering duration, but the frequencies of the parental classes in the F_2 would seem to indicate that the number of factors involved cannot be more than three. There is no definite transgressive variation in the F_2, the range just covering the two parental ranges.

The next two cases to be considered are rather complicated, the flowering duration being controlled by multiple factors but still conforming to a Mendelian interpretation. The cases 4 and 5 refer to 2 sets of crosses done on the station years ago for the study of the inheritance of rice colour. T. 102 is a variety obtained from Burma with several deeply pigmented vegetative parts and with black glutinous rice. This was crossed on to two other varieties T. 29 and T. 6, the former with ordinary red rice and the latter with ordinary white rice.

4. Cross T. 102×T. 29.

The average flowering duration of the parents were 101 and 92 days respectively. Since the cross was not primarily designed for the study of the flowering duration, the character was not noted in F_1 and F_2. Some of the F_2s were found to segregate for duration. The segregation was very definite in that the flowering was in 2 flushes with an interval, and plants could be easily counted into earlies and lates by eye judgment. The separation into earlies and lates in this cross as well as in the one to follow was made all the more easy on account of a high correlation between this character, duration, and another easily made-out quantitative character, namely, height of plant. The early plants were all short and the late plants were all tall. This point is discussed in detail in a subsequent article [Ramiah, 1933,1]. Simple counts of early and short plants and late and tall plants in two F_3 families, 2496 and 2509, gave the following ratios :—

	Early short	Late tall
2496	83	30
2509	26	9
	109	39

This ratio undoubtedly represents a 3 : 1. A large number of selections in each of these, 70 in the former and 29 in the latter, covering both the early and late groups, were carried forward and an F_4 raised therefrom. Their F_4 behaviour was as below :—

	Pure early	Splitting into early and late	Pure late
2496 group			
50 early selections	15	35	
20 late „	..	..	All pure late
2509 group			
21 early selections	8	13	
8 late „	..	..	All pure late
	23	48	

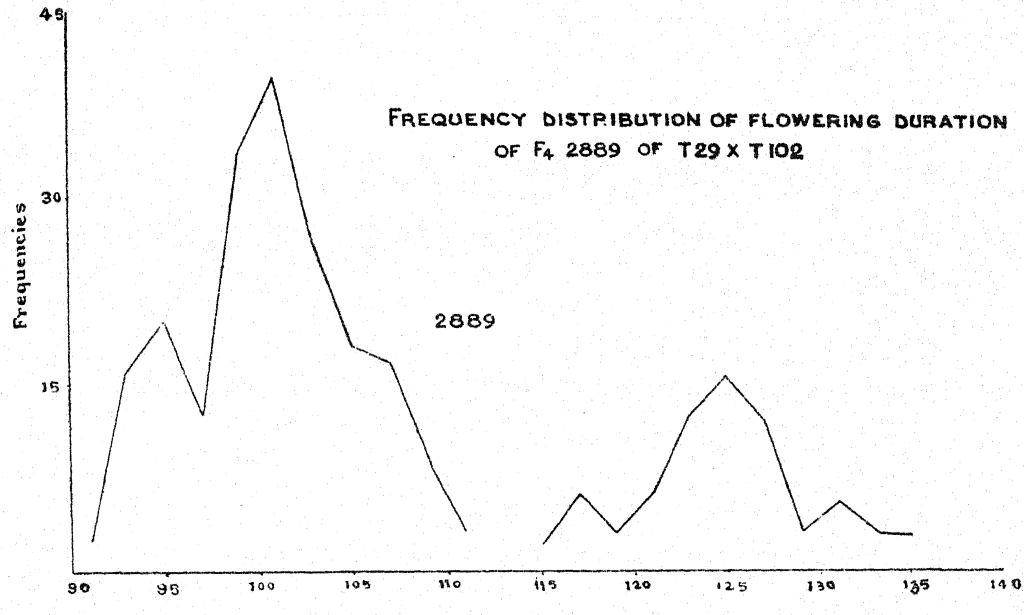


Fig. 8.

The behaviour of the F_4s thus confirmed the F_3 results, that earliness was a simple dominant to lateness. The average duration of F_3 families was found to vary from 89 to 112 days of which the average durations of 2496 and 2509 had been noted as 89 and 93 days respectively. Evidently both belonged to the early group and hence were heterozygous for the character, flowering duration. The frequencies of all the splitting families gave a bimodal curve with a clear break in the middle. Fig. 8 represents the curve of a typical splitting family, and Tables VIII and IX give the complete data regarding the two groups. The 48 segregating families together gave 5677 earlies and 1963 lates, a very close 3 : 1 ratio.

TABLE VIII.

Flowering data of F_4 families of cross T. 29×T. 102, 2496 group.

Family number	Character of parent	PURE EARLIES		SPLITTING INTO EARLIES AND LATES			PURE LATES	
		Period of flowering (days)	Mean duration (days)	Period of flowering (days)	Earlies	Lates	Period of flowering (days)	Mean duration (days)
2804	Early	83-98	92	..	..	..	..	..
2805	,,	83-98	92	..	..	..	..	..
2806	,,	94-106	98	..	..	..	..	..
2807	,,	83-100	93	..	..	..	..	..
2808	,,	83-98	93	..	..	..	..	..
2809	,,	87-101	94	..	..	..	..	..
2811	,,	90-102	97	..	..	..	..	..
2812	,,	91-108	98	..	..	..	..	..
2813	,,	83-97	92	..	..	..	..	..
2814	,,	90-106	95	..	..	..	..	..
2817	,,	94-108	98	..	..	..	..	..
2821	,,	91-100	..	..	..	..	..	..
2830	,,	93-108	100	..	..	..	..	..
2849	,,	89-110	97	..	..	..	..	..
2853	,,	94-108	102	..	..	..	..	..
2803	,,	..	..	93-123	72	34	..	..
2810	,,	..	..	89-118	123	34	..	..
2815	,,	..	..	92-117	83	20	..	..
2816	,,	..	..	95-123	71	28	..	..
2818	,,	..	..	91-118	65	25	..	..
2819	,,	..	..	91-119	81	24	..	..
2820	,,	..	..	90-118	69	26	..	..
2822	,,	..	..	89-122	149	47	..	..
2823	,,	..	..	87-117	83	21	..	..
2824	,,	..	..	93-128	191	61	..	..
2825	,,	..	..	90-119	77	29	..	..
2826	,,	..	..	83-115	78	29	..	..
2827	,,	..	..	91-120	75	30	..	..
2828	,,	..	..	89-122	254	81	..	..
2829	,,	..	..	94-120	61	28	..	..
2831	,,	..	..	104-129	140	58	..	..
2832	,,	..	..	92-124	75	20	..	..
2833	,,	..	..	92-120	71	30	..	..
2834	,,	..	..	89-119	72	31	..	..
2835	,,	..	..	83-118	77	25	..	..
2836	,,	..	..	83-113	85	20	..	..
2837	,,	..	..	89-121	88	18	..	..
2838	,,	..	..	98-125	183	67	..	..
2839	,,	..	..	89-120	182	69	..	..
2840	,,	..	..	94-123	86	23	..	..
2841	,,	..	..	91-125	75	30	..	..
2842	,,	..	..	98-126	73	27	..	..
2843	,,	..	..	94-125	83	27	..	..
2844	,,	..	..	93-124	104	42	..	..
2845	,,	..	..	89-116	78	24	..	..
2847	,,	..	..	92-125	77	25	..	..
2848	,,	..	..	94-123	76	22	..	..
2850	,,	..	..	83-122	174	65	..	..
2851	,,	..	..	98-126	68	28	..	..
2852	,,	..	..	98-128	72	31	..	..

TABLE VIII—*contd.*

Family number	Character of parent	Pure earlies		Splitting into earlies and lates			Pure lates	
		Period of flowering (days)	Mean duration (days)	Period of flowering (days)	Earlies	Lates	Period of flowering (days)	Mean duration (days)
2846	Late	..	..	..	..	..	110-124	117
2854	,,	..	..	..	..	..	112-128	122
2855	,,	..	..	..	..	..	106-122	114
2856	,,	..	..	..	..	..	104-123	113
2857	,,	..	..	..	..	..	105-122	113
2858	,,	..	..	..	..	..	106-121	112
2859	,,	..	..	..	..	..	109-124	116
2860	,,	..	..	..	..	..	110-124	118
2861	,,	..	..	..	..	..	108-126	117
2862	,,	..	..	..	..	..	108-122	115
2863	,,	..	..	..	..	..	113-126	119
2864	,,	..	..	..	..	..	110-127	118
2865	,,	..	..	..	..	..	109-126	117
2866	,,	..	..	..	..	..	110-128	119
2867	,,	..	..	..	..	..	114-130	121
2868	,,	..	..	..	..	..	108-124	115
2869	,,	..	..	..	..	..	110-128	118
2870	,,	..	..	..	..	..	110-127	118
2871	,,	..	..	..	..	..	106-125	114
2872	,,	..	..	..	..	..	112-128	120
Total number in each group		15		35			20	
Total ratios of early to late in splitting families . .					3,471	1,199	Dev./S. E. = 1·1	
Calculated 3 : 1					*3,502·5*	*1,167·5*		

TABLE IX.

Flowering data of F_4 families of Cross T. 293×T. 1359, 2509 group.

Family number	Character of parent	Pure earlies		Splitting into earlies and lates			Pure lates	
		Period of flowering (days)	Mean duration (days)	Period of flowering (days)	Earlies	Lates	Period of flowering (days)	Mean duration (days)
2873	Early	90-102	97	..	..	..	..	..
2874	,,	88-108	98	..	..	..	..	..
2875	,,	90-106	96	..	..	..	..	..
2878	,,	94-112	102	..	..	..	..	..
2882	,,	93-107	98	..	..	..	..	..
2883	,,	87-105	94	..	..	..	..	..
2884	,,	90-104	97	..	..	..	..	..
2887	,,	90-106	97	..	..	..	..	..

TABLE IX—*contd.*

Family number	Character of parent	PURE EARLIES		SPLITTING INTO EARLIES AND LATES			PURE LATES	
		Period of flowering (days)	Mean duration (days)	Period of flowering (days)	Earlies	Lates	Period of flowering (days)	Mean duration (days)
2876	Early	..	..	87-128	62	38	..	..
2877	,,	..	..	89-136	81	24	..	..
2879	,,	..	..	90-138	207	63	..	..
2880	,,	..	..	87-139	384	143	..	..
2881	,,	..	..	91-137	434	116	..	..
2885	,,	..	..	91-131	75	29	..	..
2886	,,	..	..	90-132	87	21	..	..
2888	,,	..	..	90-128	372	150	..	..
2889	,,	..	..	91-134	194	70	..	..
2890	,,	..	..	90-138	78	22	..	..
2891	,,	..	..	90-124	78	31	..	..
2892	,,	..	..	93-137	84	25	..	..
2893	,,	..	..	91-127	73	32	..	..
2894	Late	..	..	..	..	..	118-144	131
2895	,,	..	..	..	..	..	117-137	124
2896	,,	..	..	..	..	..	131-143	136
2897	,,	..	..	..	..	..	120-139	126
2898	,,	..	..	..	..	..	118-129	128
2899	,,	..	..	..	..	..	119-139	130
2900	,,	..	..	..	..	..	132-152	140
2901	,,	..	..	..	..	..	120-140	130
Total number in each group		8		13			8	

Total ratios of early to late	2,209	764	$\frac{\text{Dev.}}{\text{S. E.}} = 1{\cdot}1$
Calculated 3 : 1	*2,230*	*743*	

The average mean duration of the pure earlies of the two groups, 2496 and 2509, is found to be 96 and 99 days respectively, both definitely below the mean of the early parent. Examining the mean duration of the pure lates in 2496 group, it is either the same as that of the late parent or definitely greater than the same. As regards 2509 group, the mean duration of the lates are all definitely very much in excess of the mean of the late parent. Comparing the ranges of the splitting families in the two groups, those of 2509 group have definitely wider range than those of 2496. In spite of the varying ranges in the two groups the simple segregation into earlies and lates is very definite in both the groups. It is quite possible that the two groups, though the same for the simple split, may be different in their genetic composition. This point will be considered later when the factorial interpretation is taken up.

5. *Cross T. 102×T. 6.*

The mean durations of the parents were 101 and 84 days, with a difference of about two weeks, T. 6 being the earlier. The F_1 has been noted to have been intermediate in duration between the two parents. No detailed studies were made in F_2 regarding flowering durations. Fifteen selections made in one of the F_2 families were studied for duration in F_3. As in the previous cross there was a definite segregation into early and late in some of these, while others bred pure either for earliness or for lateness. Of the 15 selections, 5 were pure earlies, 3 were pure lates and 7 gave both earlies and lates, in the proportion of 1 : 3, the converse of the previous cross. The data regarding these F_3 families are given in Table X.

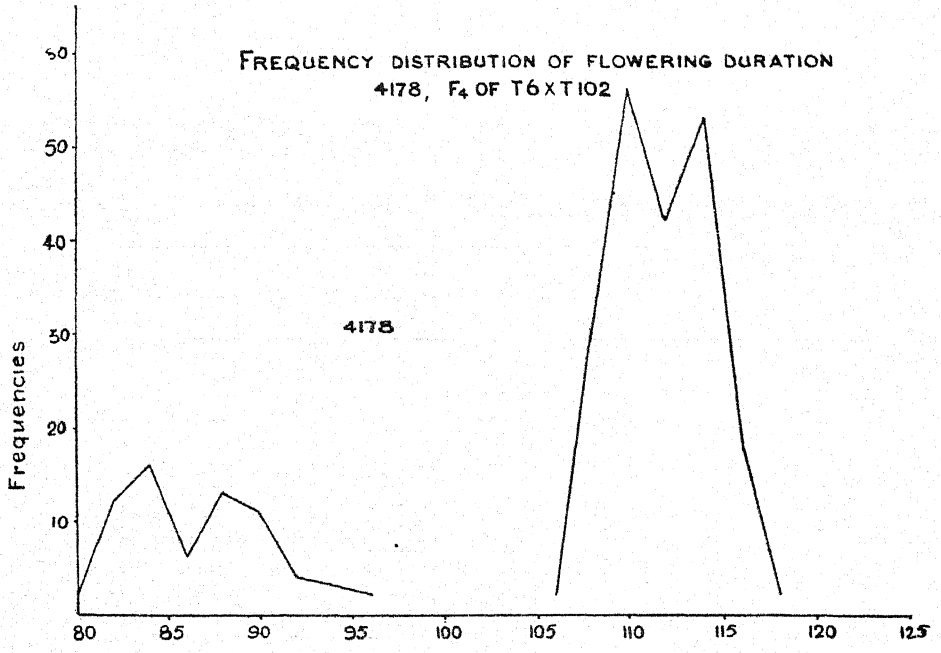
TABLE X.

Flowering data of the F_3, F_4 and F_5 of T. 102×T. 6.

Generation	Serial number	Character of parent	PURE EARLIES		SPLITTING INTO EARLIES AND LATES			PURE LATES	
			Period of flowering (days)	Mean duration (days)	Period of flowering (days)	Earlies	Lates	Period of flowering (days)	Mean duration (days)
F_3	2703	..	88-110	101	..	..	..	..	..
	2705	..	88-116	96	..	..	..	..	..
	2707	..	88-100	94	..	..	..	..	..
	2709	..	74-90	94	..	..	..	..	..
	2713	..	80-96	89	..	..	..	..	..
	2699	..	..	..	84-127	25	120	..	..
	2700	..	..	..	80-124	102	378	..	..
	2701	..	..	..	78-120	42	153	..	..
	2704	..	..	..	75-122	43	132	..	..
	2708	..	..	..	77-122	74	218	..	..
	2711	..	..	..	77-117	83	320	..	..
	2712	..	..	..	75-115	23	60	..	..
	2714	..	..	..	Split not done			..	..
	2702	..	..	..	..	..	..	100-122	111
	2706	..	..	..	..	..	..	96-114	110
F_4	3381	..	..	..	..	116	349	..	..
F_5	4169	Early	80-98	85	..	..	..	..	..
	4170	,,	80-94	87	..	..	..	..	..
	4171	,,	80-96	84	..	..	..	..	..
	4172	,,	78-96	83	..	..	..	..	..
	4174	Late	..	..	78-118	97	254	..	..
	4176	,,	..	..	78-118	68	234	..	..
	4177	,,	..	..	80-118	79	349	..	..
	4178	,,	..	..	80-118	69	203	..	..
	4179	,,	..	..	78-114	75	178	..	..
	4180	,,	..	..	84-118	47	140	..	..
	4173	,,	..	..	..	..	..	106-118	113
	4175	,,	..	..	..	..	..	104-116	112
						943	3,088		
				Calculated 1 : 3		1,008	3,023		

Early parent 84
Late parent 101

The total ratios of earlies to lates give a fair 1 : 3 ratio. Examining the ranges of pure earlies and pure lates in F_3, F_4, and F_5s, we find that they all have a greater range and a higher mean duration than the parents. The ranges of the heterozygous families cover the combined ranges of the pure earlies and pure lates and go even a little beyond the *plus* extreme of the late parent. Fig. 9 gives the frequencies of a typical splitting family.



Flowering duration—days.

Fig. 9.

The results of the two crosses, 4 and 5, above described may now be explained with the help of 3 Mendelian factors. Of the 3 parents involved in the 2 crosses T. 6 is very early, T. 102 slightly later. Let us assume that two factors $\mathbf{E_1}$ and $\mathbf{E_2}$ control earliness slightly differing in effect and one factor L_1 controls lateness, and also $\mathbf{E_1}$ is dominant to L_1 and L_1 is dominant to $\mathbf{E_2}$. L_1 can thus have no effect when $\mathbf{E_1}$ is present, and $\mathbf{E_2}$ can have no effect when L_1 is present. The parents can then be denoted by the formulæ.

T. 29—$\mathbf{L_1L_1E_1E_1e_2e_2}$—medium early.

T. 102—$\mathbf{L_1L_1e_1e_1E_2E_2}$—medium late.

T. 6—$\mathbf{l_1l_1e_1e_1e_2e_2}$—very early.

The first cross T. 29 × T. 102 would thus be $L_1L_1E_1E_1e_2e_2 \times L_1L_1e_1e_1E_2E_2$ and the cross T. 102×T. 6 would be $L_1L_1e_1e_1E_2E_2 \times l_1l_1e_1e_1e_2e_2$. Both the crosses would conform to a dihybrid ratio in the F_2. The genotypes of the F_2 and their behaviour in F_3 are outlined in Table XI.

TABLE XI.

Factorial interpretation of the crosses T. 29×T. 102 and T. 6×T. 102.

T. 29×T. 102.

T. 29	$L_1L_1E_1E_1e_2e_2$
T. 102	$L_1L_1e_1e_1E_2E_2$
F_1	$L_1L_1E_1e_1E_2e_2$

F_2 genotypes	Ratio	Character	F_3 behaviour
$L_1L_1E_1E_1E_2E_2$	1	Early	Pure early
$L_1L_1E_1E_1E_2e_2$	2	,,	All early
$L_1L_1E_1e_1E_2E_2$	2	,,	3 early to 1 late
$L_1L_1E_1e_1E_2e_2$	4	,,	As F_2, 12 early : 4 late
$L_1L_1E_1E_1e_2e_2$	1	,,	Pure early
$L_1L_1E_1e_1e_2e_2$	2	,,	3 early to 1 late
$L_1L_1e_1e_1E_2E_2$	1	Late	Pure late
$L_1L_1e_1e_1E_2e_2$	2	,,	All late
$L_1L_1e_1e_1e_2e_2$	1	,,	Pure late
Total		12 early : 4 late.	

T. 6 ×T. 102.

T. 6	$l_1l_1e_1e_1e_2e_2$
T. 102	$L_1L_1e_1e_1E_2E_2$
F_1	$L_1l_1e_1e_1E_2e_2$

F_2 genotypes	Ratio	Character	F_3 behaviour
$L_1L_1e_1e_1E_2E_2$	1	Late	Pure late
$L_1l_1e_1e_1E_2E_2$	2	,,	3 late to 1 early
$L_1L_1e_1e_1E_2e_2$	2	,,	All late
$L_1l_1e_1e_1E_2e_2$	4	,,	As F_2, 12 late : 4 early
$L_1L_1e_1e_1e_2e_2$	1	,,	Pure late
$L_1l_1e_1e_1e_2e_2$	2	,,	3 late : 1 early
$l_1l_1e_1e_1E_2E_2$	1	Early	Pure early
$l_1l_1e_1e_1E_2e_2$	2	,,	All early
$l_1l_1e_1e_1e_2e_2$	1	,,	Pure early
Total		12 late : 4 early.	

The dominant earlies of T. 29×T. 102 may be either $L_1L_1E_1E_1E_2E_2$ or $L_1L_1E_1E_1e_2e_2$ in constitution and it is quite possible there may be slight differences between the two. So also the lates may be either $L_1L_1e_1e_1E_2E_2$ or $L_1L_1e_1e_1e_2e_2$. It is quite likely that the latter may be later than the former, on account of the absence of E_2 which should counteract the late factor L_1. It was mentioned before that in the two F_2 groups of the cross T. 102×T. 29 some of the pure recessive lates of the 2496 group were like the late parent, while others were even later. If the 2496 family which gave rise to the F_4 selections had been of the composition of $L_1L_1E_1e_1E_2e_2$, of the lates resulting from it some would be $L_1L_1e_1e_1E_2E_2$ resembling the late parent in duration, others would be $L_1L_1e_1e_1e_2e_2$ which would be later than the late parent. Similarly of the pure earlies resulting from this F_3, a great number would be $L_1L_1E_1E_1E_2E_2$ and a small number would be $L_1L_1E_1E_1e_2e_2$. The former on account of the presence of both the early factors could be earlier than the early parent while the latter would have just the same duration as the early parent. On the same principle, if the F_3 family 2509 is taken to have been of the composition $L_1L_1E_1e_1e_2e_2$, the earlies $L_1L_1E_1E_1e_2e_2$, would be of the same duration as the early parent while the lates being $L_1L_1e_1e_1e_2e_2$ would all be later than the late parent. Though both the F_3s, 2496 and 2509 were taken as earlies, their constitution might have been $L_1L_1E_1e_1E_2e_2$ and $L_1L_1E_1e_1e_2e_2$ respectively.

Under the same reasoning in the cross T. 6×T. 102 the pure lates and the pure earlies will be different in composition. Since the families 2699-2714 are all F_3s, the parent F_2 should have been of the composition $L_1l_1e_1e_1E_2e_2$. This should give two kinds of pure earlies, a larger number of $l_1l_1e_1e_1E_2E_2$ and a smaller number of $l_1l_1e_1e_1e_2e_2$. This may account for the absence of any pure early of the early parental duration ($l_1l_1e_1e_1e_2e_2$). Nothing can be said about the pure lates as a sufficient number had not been grown. The F_4, 3381 which gave rise to the F_5s, 4169-4180, should have been of the constitution $L_1l_1e_1e_1e_2e_2$. The pure earlies from this would all be $l_1l_1e_1e_1e_2e_2$ and hence resemble the early parent in duration. The pure lates out of this would all be $L_1L_1e_1e_1e_2e_2$ which on account of the absence of E_2 would be later than the late parent, $L_1L_1e_1e_1E_2E_2$. Thus all the observed facts fit in with the assumptions admirably.

The results of these two crosses can be explained with the help of two factors also as below. **L** is a factor for lateness and **I** an inhibitory factor which inhibits **L** and makes it early. **L** in the absence of **I** will be late and **l** whether L is present or not will be early. The three parents will then be represented by :—

T. 29—**lL**, early ; T. 102—**iL**, late ; T. 6—**il**, early.

Cross T. 29×T. 102 should give three **IILL** (early) to one **iill** (late). Similarly T. 6×T. 102 should give 3 **iiLL** (late) to one **iill** (early). Though this interpretation is much simpler, it cannot satisfactorily explain the occurrence of types earlier than the early parent and types later than the late parent.

Since these crosses had proved very interesting both for their definite splits for flowering duration and for the markedly high positive correlation between the time of flowering and the height of plants, pure types of earlies and lates were extracted from the above sets of crosses with a view to cross them again, and these crosses are dealt with in a subsequent article [Ramiah, 1933, 2].

6. *Cross T. 24 × T. 280.*

T. 24 has been used in several of the crosses dealt with earlier and is one of the standard strains with a flowering duration of about 100 days under normal conditions, varying with different seasons according to the time of sowing. The flowering duration of T. 280 varies from 134 to 148 days. Unlike several other late varieties the ears do not all come out and finish in a flush but have a dragging characteristic. The difference in flowering time between the two varieties is about 5 weeks, the largest difference we have had in any of the crosses hitherto dealt with.

The cross was first made on 3 plants, *a*, *b*, and *c*, in each of the parents. The F_1s were grown along with the two parents on either side. The flowering duration of the parents and the F_1s are given below :—

		F_1 family number	Mean flowering duration in days
T. 24/a × T. 280/*a*	T. 24	..	102
	T. 280	..	134
	F_1	2106	109
T. 24/b × T. 280/*b*	T. 24	..	101
	T. 280	..	142
	F_1	2107	115
T. 24/c × T. 280/*c*	T. 24	..	101
	T. 280	..	137
	F_1	2108	109

The duration of the F_1 cannot strictly be said to be intermediate but is certainly more inclined towards the early parent indicating that the factors for earliness may be predominating. A number of F_2s was grown in the following season along with the parents. Among other characters studied in F_2, the flowering time of individual plants was recorded in 8 families, 6 out of F_1, 2107, and 2 out of F_1, 2108. It was observed that the F_2s started flowering a week or two earlier than the early parent, T. 24, and continued the phase several weeks. There was a fairly rapid flush of flowering corresponding to the time of flowering of the early parent with the maximum frequency before the 4th week from the commencement. After this period, however, there was a gradual fall up to the 13th week corresponding to the flowering time of the late parent, T. 280. Beyond this there was no regular flowering at all, only stray plants putting forth just one head only without any signs of the whole plant finishing the flowering phase. This went on for another 3 or 4 weeks and after 18 weeks from the commencement of flowering, there were still a good number of plants in each family growing continuously without any sign of producing panicles. At the end of the season, these were topped and the stubbles retransplanted. These stubbles grew again but did not produce any earheads even during the 2nd season. This peculiarity was found to be a constant feature of all the F_2 families. On careful examination of the frequencies it was found that the total of all the plants that finished flowering in the main flush, gave to the rest, the totals of a few stray indifferently flowering plants after the 13th week together with the absolutely non-flowering late ones, a good 15 : 1 ratio. This ratio was quite definite in all the 8 F_2 families (Table XII). The flowering durations of the two parents are also indicated in the table. As was stated already the flowering period of T. 280, the late parent, was protracted extending to nearly 3 weeks. Although the flowering of plants was recorded every day, the frequencies as given in Table XII and Fig. 10 are per week, obtained by adding up the daily records. No selections were made in any of the F_2s but in the following season, seed of two more F_1 plants, which had been preserved in the previous season were sown to get confirmatory results about this flowering duration. To see whether the non-flowering lates would flower if the seeds were sown earlier, these F_2s were sown quite a month in advance of the usual sowing time. The plants started flowering nearly a month earlier than in the previous season and even here, the few lates which put forth stray heads together with the non-flowering lates make to the rest a 1 : 15 ratio (bottom of Table XII). On account of the very early sowing the parental durations did not conform to the general average, particularly in the case of the early variety T. 24. In spite of the difference in season it is found that the maximum frequency is on the 3rd week as in the case of the previous 8 families.

TABLE XII.

Flowering frequencies of the F_2s of T. 24 × T. 280.

	Flowering frequencies by week													Total	Very lates stray flowering beyond 13th week	Non-flowering lates	Total
	1	2	3	4	5	6	7	8	9	10	11	12	13				
1st season sown 29th July.																	
Parent T. 24	..	16	30	..	..	..	..	..	..	..	..	..	..	..	...	..	..
„ T. 280	..	..	..	..	..	..	..	5	39	5	..	..	..	..	..	..	..
F_2 numbers—flowering commencing from 1st November.																	
2610	33	305	564	405	221	143	148	137	120	66	42	2	9	2,195	18	114	132
2611	10	172	325	211	115	72	85	56	48	30	20	5	4	1,153	10	62	72
2612	16	164	335	209	104	95	75	50	43	32	13	5	4	1,145	15	55	70
2613	7	193	290	261	118	98	102	53	52	36	29	1	4	1,139	14	52	66
2614	5	77	276	228	127	123	90	46	53	34	22	2	2	1,085	16	53	69
2615	36	320	498	294	134	97	132	86	39	15	13	3	4	1,671	36	78	114
2616	56	225	267	191	126	56	63	60	22	27	25	21	8	1,147	31	33	64
2617	19	139	264	212	117	45	74	40	36	22	20	20	6	1,014	36	46	82
Total for 8 families =														10,549	..	..	669 Dev. / 701 S.E. = 1·3
Calculated 15 : 1 =														10,517	..	..	
2nd season—sown 24th June.																	
Parent T. 24	..	.	10	17	..	..	..	..	..	..	..	..	..	..	..	..	..
„ T. 280	..	..	..	..	7	6	9	..	..	..	..	..	..	..	..	..	..
F_2 numbers—flowering commencing from 1st October.																	
2723	19	174	284	238	170	95	68	59	29	14	6	12	23	1,191	20	48	68
2724	22	144	232	183	150	78	48	64	26	20	14	5	15	1,001	14	38	52
Total for 2 families =														2,192	..	..	120 Dev. / 144·5 S.E. = 2·1
Calculated 15 : 1 =														2,167·5	..	..	
Totals for all the 10 F_2 families =														12,741	..	..	789 Dev. / 846 S.E. = 2·0
Calculated 15 : 1 =														12,684	..	..	

D

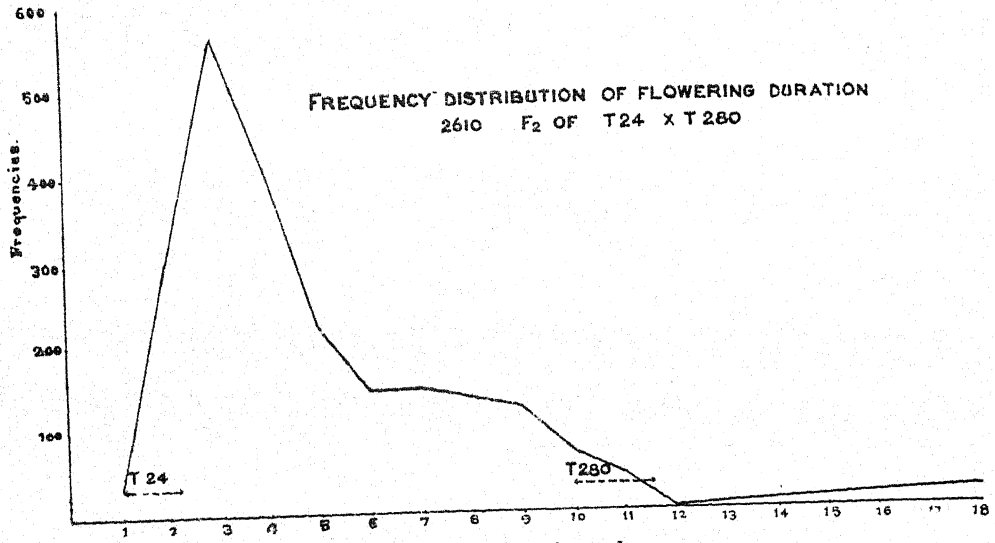


Fig. 10.

A large number of selections, about 100, were made in F_2 family, 2723, and an F_3 raised in the following season. Although the F_3 results first appeared complicated and unintelligible, definite types of segregation could be made out as below.

(*a*) Families behaving exactly like F_2, giving 15 : 1 of earlies and lates to very lates and non-flowering lates.

(*b*) Families exhibiting the same kind of segregation as (*a*) but with 3 : 1 ratio instead of 15 : 1 as in the former.

(*c*) A good number of families which did not throw any lates apparently pure for earliness. These were of three kinds (1) those having a smaller mean duration than T. 24, (2) those having mean duration about the same or slightly greater than T. 24, and (3) those having mean durations definitely greater than T. 24.

(*d*) Families having a range starting with that of the late parent and going very much beyond the same with a very large number of non-flowering lates, and this non-flowering lates to the rest making a ratio of 3 : 1.

(*e*) Families having a range starting beyond the *plus* extreme of the late parent and with almost all the plants remaining non-flowering.

From the fact that the F_1 was very early, and in the F_2 there was an indication of a 15 : 1 ratio of earlies to lates and that the same is repeated in a good number of F_3s, it is highly probable that the factors concerned in the inheritance of flowering duration in this cross cannot be too many. Probably only 3 factors are involved and, as is shown below, a three-factor hypothesis appear to agree with the results

obtained in F_2s and F_3s. Let us assume that (1) $\mathbf{E_3}$ is a factor for earliness, (2) $\mathbf{L_2}$ is a factor for lateness which by itself makes the plants extremely late and they do not flower normally, (3) $\mathbf{E_4}$ is another factor for making the non-flowering lates to flower, thus counteracting the effect of $\mathbf{L_2}$ though these flower very much later than the early parent, and (4) $\mathbf{E_3}$ is dominant to $\mathbf{L_2}$ and $\mathbf{E_4}$. With the above assumptions the two parents will have the formulæ :—

T. 24 early parent . $\mathbf{E_3E_3e_4e_4l_2l_2}$.
T. 280 late parent . $\mathbf{e_3e_3E_4E_4L_2L_2}$.

For purposes of description the families which are all definitely earlier than the late parent will be termed earlies, those that are of the same duration as T. 280 as lates, those that are later than T. 280 but still flower irregularly as very lates, and those that do not flower at all as non-flowering lates. As per the formulæ suggested above, the F_1 would be $\mathbf{E_3e_3E_4e_4L_2l_2}$. The F_2s should conform to a trihybrid ratio. Table XIII which gives the constitutions of the F_2 types and their F_3 behaviour agree fairly with the expectations. For such a complicated character as flowering duration which varies from season to season due to environment, even with the assumption of 3 factors, it should be very difficult to make an accurate classification of the F_3 types. It is likely that all the possible genotypes were not represented in the 100 F_3 families studied and there were also a few whose behaviour did not strictly conform to the interpretation suggested.

TABLE XIII.

Factorial interpretation of Cross T. 24 × T. 280.

T. 24 . $\mathbf{E_3E_3e_4e_4l_2l_2}$.
T. 280 . $\mathbf{e_3e_3E_4E_4L_2L_2}$.
F_1 . $\mathbf{E_3e_3E_4e_4L_2l_2}$.

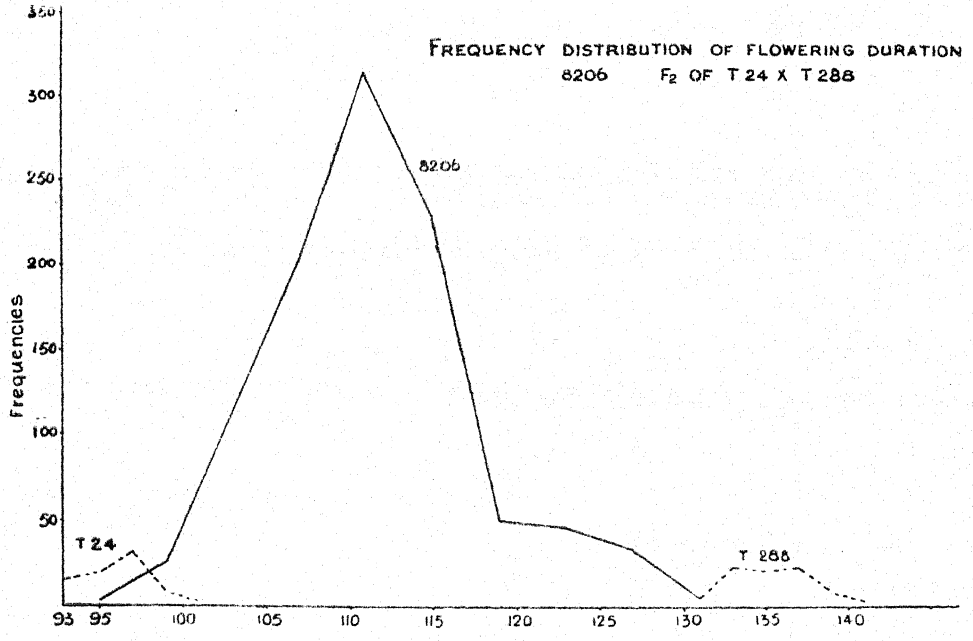
F_2 Genotypes	Ratio	Character	F_3 behaviour
$\mathbf{E_3E_3E_4E_4L_2L_2}$.	1	Early	Pure early
$\mathbf{E_3e_3E_4E_4L_2L_2}$.	2	,,	Early to late varying up to the duration of T. 280
$\mathbf{E_3E_3E_4e_4L_2L_2}$.	2	,,	Pure early
$\mathbf{E_3E_3E_4E_4l_2L_2}$.	2	,,	,, ,,
$\mathbf{E_3e_3E_4e_4L_2L_2}$.	4	,,	Early + late : non-flowering late = 15 : 1
$\mathbf{E_3E_3E_4e_4L_2l_2}$.	4	,,	Pure earlies, varying, none reaching the duration of T. 280
$\mathbf{E_3e_3E_4E_4L_2l_2}$	4	,,	Mostly earlies and some lates like T. 280
$\mathbf{E_3e_3E_4e_4L_2l_2}$.	8	,,	As F_2
$\mathbf{E_3E_3E_4E_4l_2l_2}$.	1	,,	Pure early
$\mathbf{E_3E_3E_4e_4l_2l_2}$.	2	,,	,, ,,
$\mathbf{E_3e_3E_4E_4l_2l_2}$.	2	,,	Earlies of varying duration

F_3 Genotypes	Ratio	Character	F_3 behaviour
$E_3e_3E_4e_4l_2l_2$	4	Early	Earlies of varying duration : very lates = 15 : 1
$E_3E_3e_4e_4L_2L_2$	1	,,	Pure early
$E_3E_3e_4e_4L_2L_2$	2	,,	,, ,,
$E_3e_3e_4e_4L_2l_2$	2	,,	Early : non-flowering late = 3 : 1
$E_3e_3e_4e_4L_2l_2$	4	,,	Early : very late + non-flowering = 3 : 1
$e_3e_3E_4E_4L_2L_2$	1	Late	Pure late of T. 280 duration
$e_3e_3E_4e_4L_2L_2$	2	,,	Late : non-flowering late = 3 : 1
$e_3e_3E_4E_4L_2l_2$	2	,,	Late of T. 280 duration and less
$e_3e_3E_4e_4L_2l_2$	4	,,	Early + late of T. 280 duration : very late + non-flowering late = 3 : 1
$e_3e_3e_4e_4L_2L_2$	1	Non-flowering late	Pure non-flowering late (not obtainable)
$e_3e_3e_4e_4L_2l_2$	2	,,	Very late : non-flowering late = 1 : 3
$e_3e_3E_4E_4l_2l_2$	1	Early	Pure probably later than T. 24
$e_3e_3E_4e_4L_2L_2$	2	,,	Early : very late = 3 : 1
$E_3E_3e_4e_4l_2l_2$	1	,,	Pure early as T. 24
$E_3e_3e_4e_4l_2l_2$	2	,,	Early : very late = 3 : 1
$e_3e_3e_4e_4l_2l_2$	1	Very late	Pure very late—representing group of plants flowering between the 14th-18th week.

Total=51 early ; 9 late ; 3 non-flowering late ; and 1 very late. Or early+late : non-flowering late+ very late = 60 : 4 ; or 15 : 1.

Another case of complicated inheritance with regard to flowering duration was obtained in the crosses made on the station to evolve a type combining high yield and resistance to paddy blast, *Piricularia oryzæ*. A number of crosses were made between selected apparently resistant varieties and one of the high yielding types particularly susceptible to the disease. There were variations in the flowering durations of the parents for the different crosses. Among other studies made in F_1s and F_2s flowering durations were also recorded. It was observed that the F_1 was always intermediate in duration between the two parents, slightly inclining towards the early parent. There was no apparent segregation for flowering duration in any of the F_2s and the flowering frequencies were not analysable. The ranges of the F_2s were found to start in every case with the *minus* end of the early parent and to reach up to the *minus* end of the late parent and in some cases to reach the mean of the late parent. In no case, however, did the F_2 range exceed the mean of the late parent. Fig. 11 represents graphically the flowering frequencies of the two parents and the F_2 (8206), of one of these crosses. The whole of this F_2 family was carried forward and grown as an F_3 planting about 100 seedlings to each F_3 family, and the average duration of each family recorded again. Out of 1,157 families thus studied, it was found that there were just 19 families which somewhat resembled the early parent in their ranges while there was none with a range corresponding to that of the late parent. This is analogous to the results

obtained by Emerson and East [1913] in maize. Although the number of types similar to the early parent, 19 out of 1,157 would very roughly correspond to a trihybrid ratio, the fact that not a single type corresponding to the late parent was obtained probably indicates that the number of factors concerned must be more than 3.



Flowering duration—days.

Fig. 11.

VI. Summary.

The flowering duration of rices varies enormously according to the variety from as low a figure as 60 days to as high a figure as 180 days. Some of the environmental conditions that influence the flowering duration are the time of sowing, the spacing given to the plants and the fertility of the transplant field. Though influenced by such environmental conditions, it has been possible to trace its inheritance in a number of crosses where the two parents had been grown side by side with the offspring of the crosses every season to get comparable results.

The studies have shown that the inheritance of this important character may be quite simple in some cases showing a single factor difference between earliness and lateness, and rather complicated in others which could only be explained under the multiple-factor hypothesis. There must be several genetic factors concerned

in the inheritance of flowering duration in rice, and varieties may carry in them either a few or several of these factors. Where the number of factors involved is great, the F_1 appears to be intermediate between the parents, and F_2, we either get a transgressive variation or a variation within the parental limits and the parental types are hardly recovered. Varieties that have the same flowering duration may still differ in their factorial composition with regard to this character giving a transgressive variation in the F_2s when crosses are made between them. It has been possible to derive certain pure types from such crosses which are both earlier and later than the parents. As regards earliness and lateness, earliness is generally found to be dominant and a single case has been recorded where earliness was a recessive. From several crosses discussed in this article, it would appear, that if sufficient number of generations are raised, it is possible to trace the inheritance of the character and to interpret the results on Mendelian hypothesis and also determine the number of factors involved.

ACKNOWLEDGMENTS.

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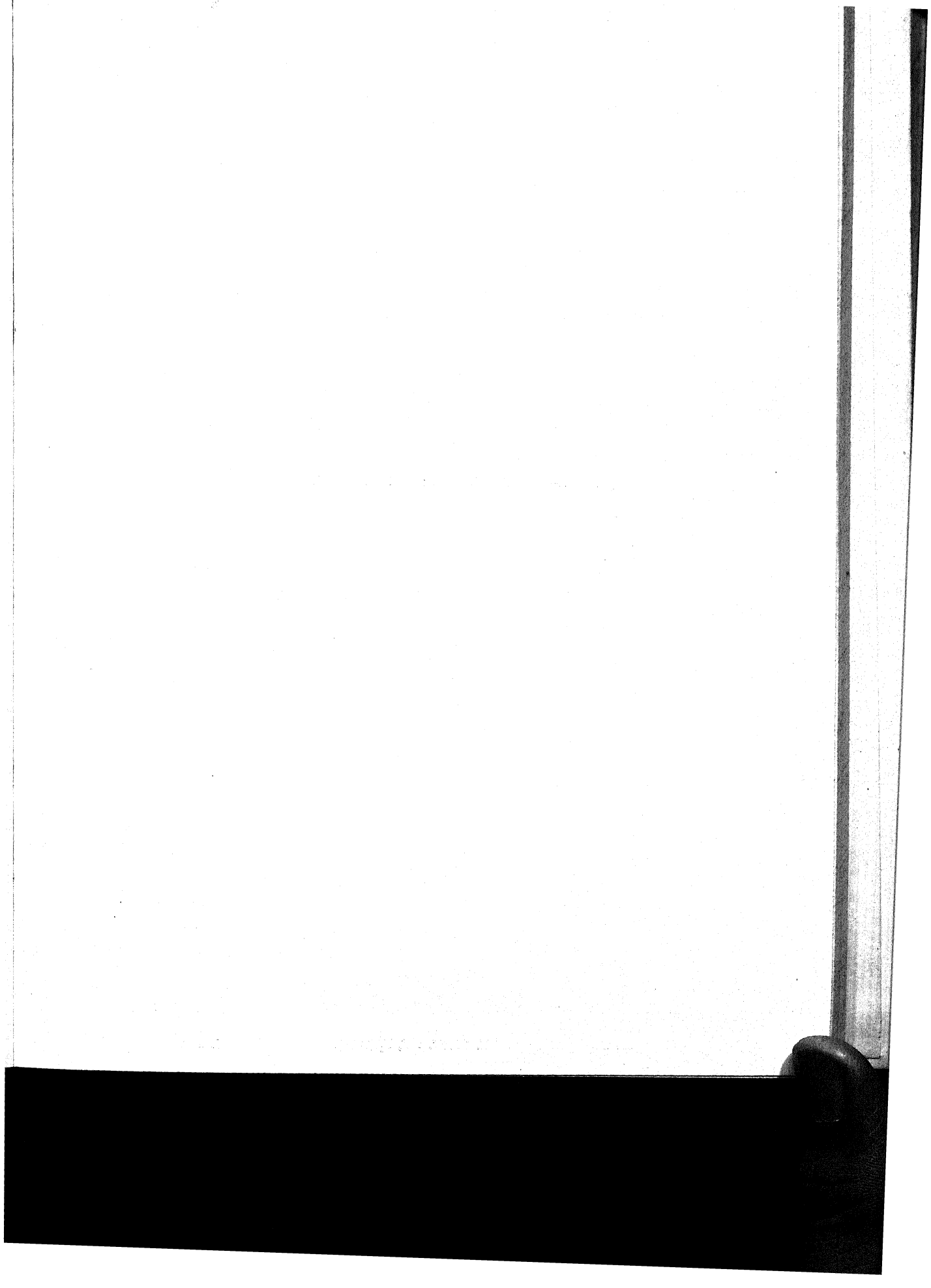


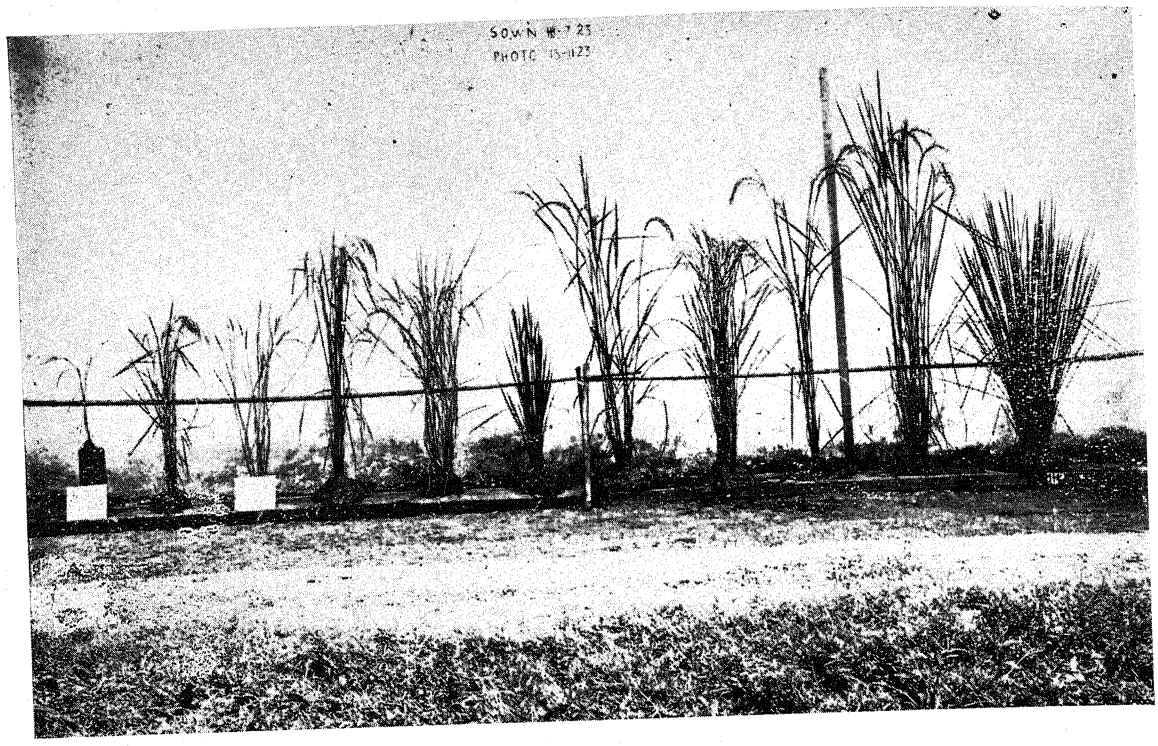
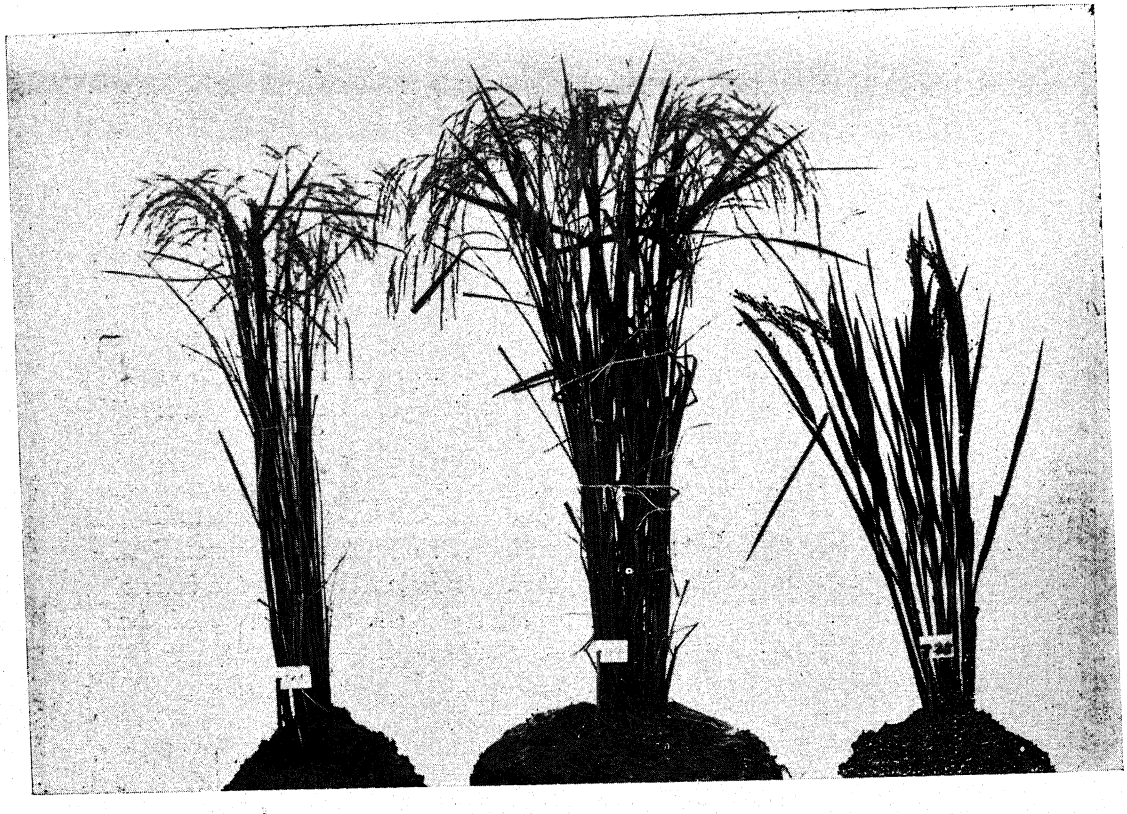


Fig. 1.—Variations in plant height of rice varieties.



T. 24. T. 24×T. 282, F_1 (3368). T. 282.

INHERITANCE OF HEIGHT OF PLANTS IN RICE (*ORYZA SATIVA L.*).

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(With Plates XXVIII-XXX and seven text-figures).

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I. Introduction.

The height of plant varies considerably in different varieties of rices. Among the pure line collections at the Paddy Breeding Station, Coimbatore, there are varieties with as short a height as 30 inches, and others as tall as 70 inches with all intermediate sizes (Plate XXVIII, fig. 1). The long-duration varieties are generally taller than those of a shorter duration with a few exceptions. The height of the plant depends upon the number of internodes and their magnitude. Though the ultimate height of a plant is subject to environmental and seasonal influences, from observations made on nearly 300 pure lines over several years, it is found that it is fairly constant from year to year. One great factor that affects the height of plant is, as in the case of flowering duration, the time of sowing. A variety that is sown very early in the season has the longest period of development and hence attains also the maximum height. As the plantings are delayed, the duration of the crop is proportionately reduced, and consequently the ultimate height of the plant is

also reduced. In illustration of this the average heights of plants in T. 24 sown at different times in a season are given below :—

Sowing time	Average height of plant in inches
July	48
August	43
September	42
October	39
November	40
December	39
January	36

There is little difference in height between varieties in the very early stages. The growth is continuous and reaches the maximum just when the plants are in the reproductive phase. The short-duration varieties, which do not generally attain a great height, grow much more rapidly than the late varieties and reach their maximum soon. On the other hand, the growth in the late varieties is continuous though slow in the beginning, and gets more and more rapid as the plants advance in age. There is no increase in height after the earheads are formed [Ramiah, 1926]. The height records are usually taken when the earheads are ripening and when the straw is just beginning to turn yellow. Each plant is held vertically with all the tillers gathered together and then a long scale with two-inch divisions is held close to the plant, the height being recorded as that point on the scale where the majority of the heads end. If the height is measured after the plants are dead ripe, there is a slight reduction from the maximum height due to the shrinkage of the straw below. If a fair number of plants are measured in any pure line, there is found to be a certain amount of variation, but the difference between the shortest and the tallest is never more than a foot in any case. The height of plants has been studied incidentally in several crosses which have been intended for the study of other characters. The present paper brings together all the recorded results in the station so far, about the inheritance of plant height. It will be seen from what follows that there have been some very simple cases, as well as cases where the character is complicated due in all probability to the interaction of the several Mendelian factors.

II. Discussion of literature.

Freeman [1919] had studied the inheritance of height in wheat and found that the factors for height were not uniform in F_2 plants. He thinks that recombination had occurred, so that, on the average tall plants gave rise to tall offspring and the grading of the parents into a series of ascending heights resulted in a

slightly less marked, but still regularly ascending series of offspring groups. The completeness of the series indicated that the number of factors was large. He concluded that all factors observed in the inheritance of height in wheat crosses are in harmony with the hypothesis of segregation of a number of simple Mendelian unit characters.

Emerson and East [1913] studied the inheritance of height in maize. Out of 4 crosses they made, the F_1 was as tall as the tall parent in three cases and in the fourth it was considerably taller than the mean of the two parents. They think that this tallness was not ascribable to its being dominant but rather to increased vigour due to heterosis. They found that all the F_2 fraternities overlapped in height the inner extremes of the parents. Most of them had a range from near the mean height of one parent to the mean height of the other parent and in one cross the F_2 ranged from the *minus* extreme of the short parent to the *plus* extreme of the tall parent. The F_3 families were very diverse in height and variability. Few F_3 families were as tall as the tall parent, though the latter was approached very closely in a few cases. In the case of some of the extreme F_3 lots, the variability was sufficient to make it probable that types like the parents could be isolated in the next generation. Moreover certain F_3 families with heights variously intermediate between the parents had variabilities small enough to indicate the possibility of their breeding true to the heights. Their results, then, generally conform to the multiple-factor hypothesis.

Ikeno [1927] has recorded that the F_1 of a cross between two rices generally approached in height the taller of the two parent plants though exceptions were not entirely excluded. In one case the height of the F_1 was intermediate between the two parents and the F_2 gave high, medium and low plants in the ratio of 1 : 2 : 1. He states that in general the inheritance of height in rice does not appear to be simple.

III. Material and methods of study.

Segregation for height of plants in any particular family is easily made out by sight. Apart from the influence of wider spacing, or an extremely fertile patch at any one point which both influence height of plants growing at that point, the genetic variation is clearly made out from the random distribution of tall and short plants throughout the plot. Fig. 1 represents the height frequencies of two families. Both are apparently normal curves, but in one the variation is within a foot whereas in the other it is about 30 inches. Evidently the one that shows the minimum variation is genetically pure for the height character while the other is not. In contrast to the above two, Fig. 2, giving the height frequencies of an F_2 family, No. 604, may be considered. There is apparently a simple dominance of

tallness to shortness, although the ratios taken on either side of the minimum frequency point are not definite. A number of selections had been made in this family for quite a different purpose and an F_3 raised. Though actual height measurements of individual plants were not made in F_3 families, simple observations had been recorded about the behaviour of the character in each of the families. Since the parental heights of each F_3 family were known, it was found that selections with heights varying from 36 to 45 inches were all pure for short height, and selections measuring 63 inches and above were pure for tall height. The selections with intermediate heights, 51 to 57 inches, have been noted to give shorts and talls. This rough analysis clearly proved that tallness and shortness formed a simple pair of allelomorphs in this cross.

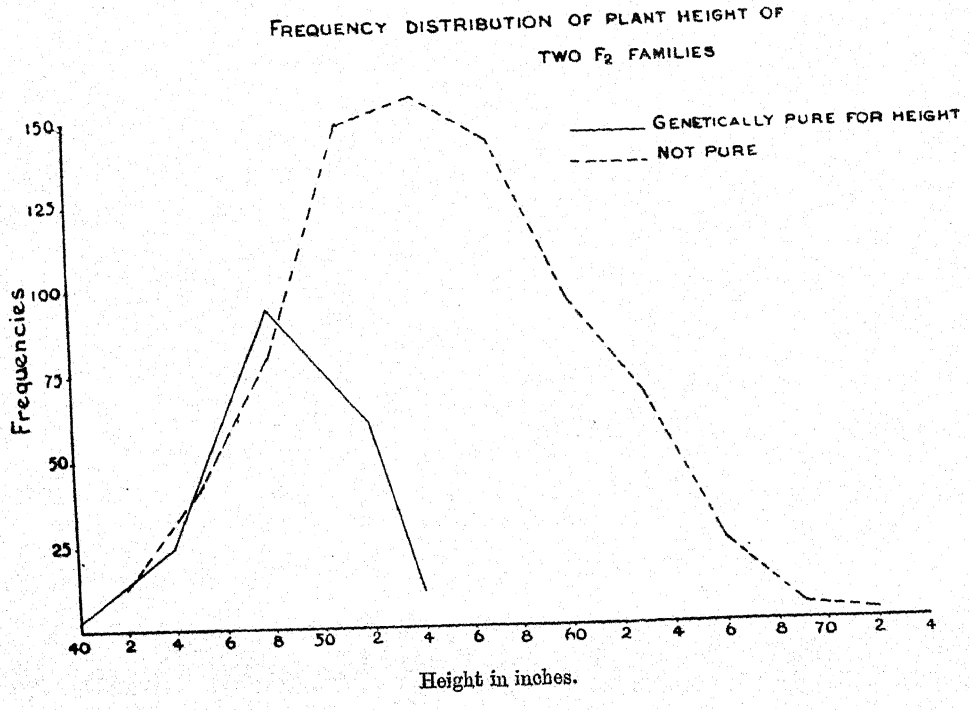


Fig. 1.

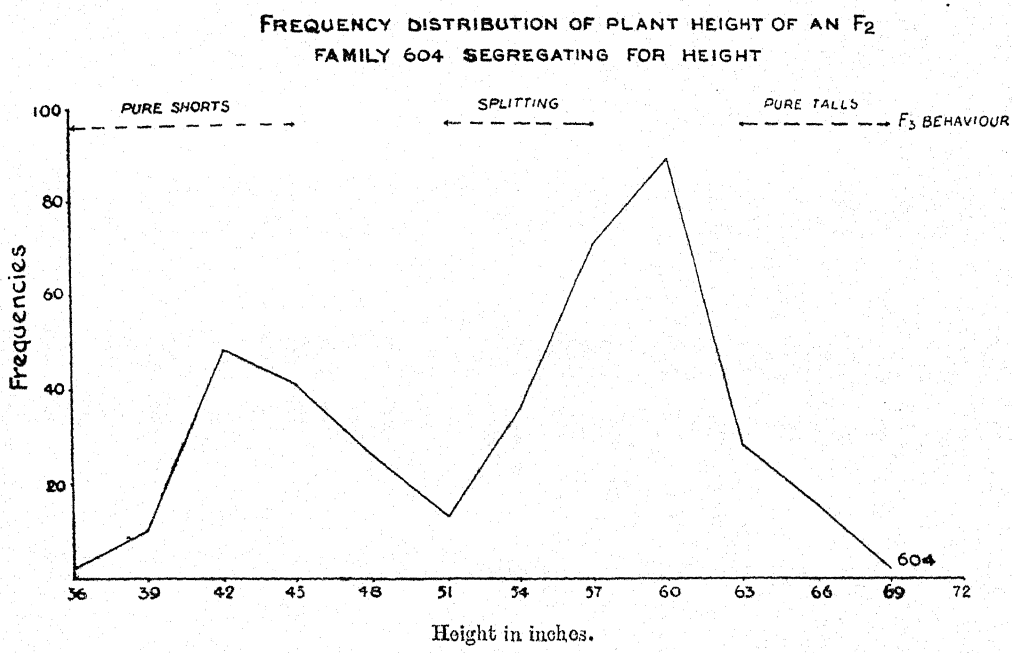


Fig. 2.

IV. Discussion of definite crosses involving height inheritance.

1. T. 24×T. 282 (a dwarf variety).

Parnell [1922] has described a definite case of dwarfness being a simple recessive to tallness. In this particular case besides the stature of the plant, all the characters of the dwarf parent, its short and compact ear, its broad leaves, its round shape of grain, etc., were all coming out together. This result was first obtained from a tall natural hybrid observed in the plot of the dwarf variety. To get confirmatory results of this behaviour an artificial cross was made between the dwarf variety and No. 24. The F_1 exhibited the almost complete dominance of tallness (Plate XXVIII, fig. 2). The height measurement data are given in Table I and the same graphically represented in Fig. 3. The height frequencies give a definite bimodal curve, the ratio between the talls and shorts being, 2029 : 437. Although the F_2 segregation was clearly a unifactorial split, the ratio between the tall and short is far out of a 3 : 1 ratio. Fifty-four selections were made from this

F_2, 34 from the tall group with varying heights and 20 from the short group and grown as F_3. Out of the 34 talls, 9 families bred pure for tallness though with varying mean heights and the other 25 gave talls and shorts again just like the F_2. The total number of talls and shorts for the 25 splitting families were :—

Talls	Shorts
3,484	1,229

The above ratio is not far out of a 3 : 1 ratio. There does not seem to be any difference in height between the homozygous and the heterozygous families. There is no sort of correlation between the F_2 parental height and the F_3 behaviour.

The 20 short selections all bred pure for shortness though with different mean heights. Even in the splitting families the mean heights of the several tall and short groups are different. Comparing the height range in the F_2 with those of the two parents, it will be seen that the two parents do not overlap each other, but in the case of the F_2 distribution, although there is a definite indication of a break with a minimum frequency, there is overlapping of the shorts and talls in height. The mean heights of the pure talls in the F_3 vary from 44·2 to 61·5 inches ; only one out of the nine pure talls has a height equal to that of the tall parent, while all the rest have a height definitely greater. The differences in the mean heights of pure talls cannot be due to mere fluctuation as they are all very significant in terms of the standard error. The segregation of the F_3s is much sharper in that there is a definite break in the height frequencies of the talls and shorts, and the two frequencies never overlap as in the case of the F_2. The frequencies on either side of the break or minimum frequency class give a good 1 : 3 ratio of short to tall. It is also seen from the table that in the splitting families there is a certain amount of uniformity in height between the tall group and the short group, their means varying together.

TABLE I.

Height data of F_2 and F_3s of T. 24×T. 282.

Generation	Family number	Character of parent and its height (inches)	Height frequencies (inches) 20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70	72	Mean height (inches) Pure shorts	Pure talls	Splitting families Shorts	Splitting families Talls	Ratio of shorts to talls Shorts	Talls
		Short parent	..	..	4	9	10	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	24·7	..	..	..	..	..
		F_2 .	..	4	11	60	107	132	88	26	16	26	88	187	392	451	388	275	144	51	15	4	1	..	..	..	..	..	..	..	..	27·5	44·5	..	..
		Tall parent	..	..	..	..	..	..	..	..	2	11	20	12	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	40·0	..	..	..	..
		Tall																																	
F_3 .	4873	48″	..	..	..	..	..	..	..	..	..	..	..	1	2	5	30	60	58	47	20	11	4	2	..	..	..	..	..	..	52·3	..	..	..	..
	4877	46″	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	15	13	18	19	12	4	2	..	..	..	..	..	57·0	..	..	..	..
	4883	54″	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	2	10	26	52	77	100	72	39	16	2	1	..	61·5	..	..	..	..
	4884	42″	..	..	..	..	..	..	..	..	..	..	..	..	1	10	22	48	42	24	6	1	..	..	..	..	..	..	..	..	50·8	..	..	..	..
	4889	50″	..	..	..	..	..	..	..	..	..	..	..	2	6	20	36	90	67	92	12	3	..	..	..	..	..	..	..	..	51·2	..	..	..	..
	4893	42″	..	..	..	..	..	..	..	1	3	7	43	90	131	138	56	11	..	..	1	..	..	..	..	..	..	..	..	..	44·2	..	..	..	..
	4894	42″	..	..	..	..	..	..	..	..	..	..	2	2	8	24	59	82	83	38	5	1	..	..	..	..	..	..	..	..	50·2	..	..	..	..
	4901	42″	..	..	..	..	..	..	..	..	..	..	2	3	6	34	62	101	39	12	2	..	..	..	..	..	..	..	..	..	49·2	..	..	..	..
	4874	44″	..	..	..	..	21	22	5	1	..	..	1	8	18	31	47	44	14	3	3	1	..	..	..	..	..	..	..	..	..	29·4	48·0	49	170
	4875	56″	..	..	..	..	..	..	..	..	1	16	1	11	4	..	..	..	1	2	16	15	27	21	15	4	1	..	..	..	..	40·0	62·0	44	104
	4876	46″	..	..	..	..	..	..	1	3	4	13	2	2	..	..	..	9	7	27	18	9	5	1	1	..	..	..	..	..	..	35·0	52·9	23	77
	4878	44″	..	..	..	..	..	..	..	1	7	4	2	2	..	..	1	1	9	21	15	7	3	..	..	..	..	..	..	..	..	35·6	54·8	16	57
	4879	46″	..	..	..	..	..	..	..	1	2	10	6	5	2	..	..	1	5	15	19	24	24	3	..	1	1	..	..	..	..	37·4	57·4	29	93
	4880	50″	..	..	..	..	..	1	..	..	10	17	27	7	1	1	1	5	6	18	31	50	30	16	4	1	..	..	..	..	..	39·0	57·6	63	163
	4881	42″	..	..	..	..	..	1	3	6	18	5	..	..	..	..	2	10	25	23	13	10	5	..	..	..	..	..	..	..	..	35·4	54·0	33	88
	4882	44″	..	..	..	..	..	..	..	..	8	14	21	6	5	1	..	1	9	17	38	33	23	15	1	2	..	..	..	..	..	37·6	55·4	55	139
	4885	46″	..	..	..	..	..	..	2	7	15	3	5	1	1	1	8	18	29	29	17	12	4	..	..	..	..	..	..	..	..	34·5	51·3	34	116
	4886	42″	..	..	1	6	9	24	18	2	..	..	..	2	11	37	60	45	27	9	2	..	..	..	..	..	..	..	..	..	..	30·1	48·7	60	193
	4887	50″	..	..	..	..	1	4	13	17	13	7	1	..	1	1	15	25	40	27	19	18	2	1	..	..	..	..	..	..	..	32·2	53·0	56	149
	4888	50″	..	..	..	..	5	21	27	19	7	1	..	2	2	2	21	45	55	59	32	7	5	..	..	..	..	..	..	..	..	31·9	52·5	80	230
	4890	52″	..	..	..	..	..	..	..	1	5	16	4	7	..	..	1	..	3	7	15	30	28	15	5	1	..	..	..	..	..	37·0	56·6	33	101
	4891	46″	..	..	..	2	1	8	19	21	10	10	2	1	..	2	7	38	47	63	35	12	1	1	..	..	..	..	..	..	..	31·8	51·2	74	206
	4892	42″	..	..	..	10	13	7	3	3	1	1	..	3	5	29	31	27	12	3	..	..	..	..	..	..	..	..	..	..	..	28·8	46·2	38	110
	4895	48″	..	..	..	4	8	17	22	17	7	1	..	..	2	15	34	56	45	29	11	5	..	..	..	..	..	..	..	..	..	31·8	50·9	76	197
	4896	44″	..	..	..	1	3	7	5	9	14	5	1	..	..	5	12	19	32	15	13	6	1	..	..	..	..	..	..	..	..	33·8	52·1	45	103
	4897	44″	..	..	..	..	4	5	11	17	12	1	2	1	..	3	8	18	28	41	18	15	2	2	..	..	..	..	..	..	..	33·5	53·4	53	135
	4898	46″	..	..	..	3	12	16	10	6	4	..	..	..	11	14	33	39	39	34	11	4	2	..	..	..	..	..	..	..	..	30·6	49·8	51	148
	4899	44″	..	..	1	1	3	18	31	34	22	6	..	..	..	9	26	45	67	70	50	20	..	..	..	..	..	..	..	..	..	33·1	52·7	116	287
	4900	36″	..	..	7	13	7	5	1	..	1	..	1	6	27	39	23	8	1	1	..	..	..	..	..	..	..	..	..	..	..	26·8	46·0	33	107
	4902	42″	..	..	..	3	13	12	5	6	..	1	1	2	9	17	39	27	15	2	1	1	..	..	..	..	..	..	..	..	..	30·0	48·4	39	115
	4903	42″	..	..	1	10	19	24	10	2	1	..	5	11	21	35	70	44	13	1	..	..	..	..	..	..	..	..	..	..	..	29·2	47·4	67	195
	4905	48″	..	..	..	..	1	2	6	18	16	6	2	..	1	1	3	16	30	53	44	21	10	3	..	..	..	..	..	..	..	34·8	54·5	67	132
	4906	54″	..	..	..	..	1	1	2	14	23	13	7	2	..	1	2	2	5	19	23	26	4	2	1	..	..	..	..	..	..	36·3	55·9	63	89

Total ratios for the 25 splitting families.

	Shorts	Talls	
	1,197	3,554	$\frac{\text{Diff.}}{\text{S. E.}} = 0{\cdot}3$
1 : 3	*1,188*	*3,563*	

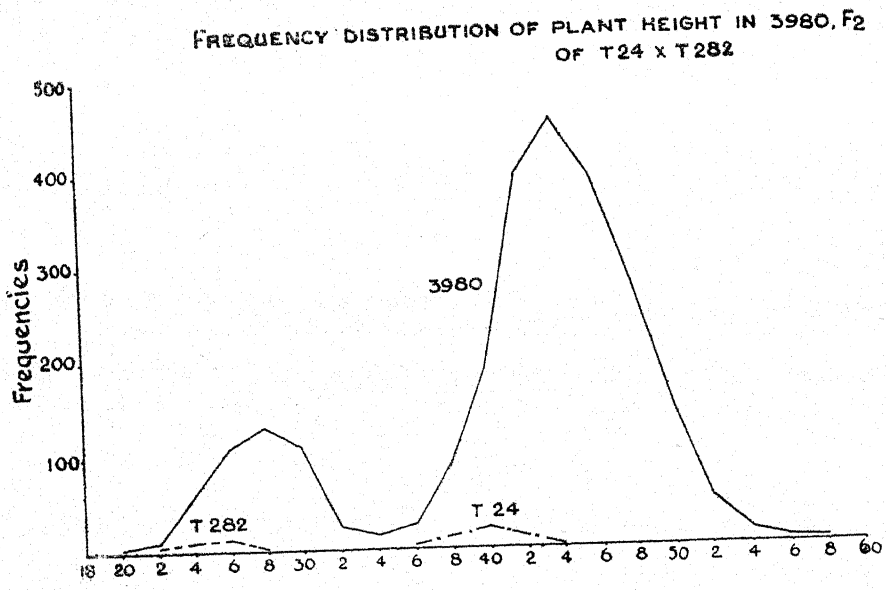


Fig. 3.

Some further selections had been made from two of the F_3 families, 4883 and 4886, and an F_4 raised. Family 4883 was one of the pure talls with a mean height of 61·5 inches. Six selections from this varied in height from 59 to 63 inches proving that they were all true to their parent. Of the 30 selections, all tall plants from family 4886 which was one of the splitting families with mean height of the tall group at 48·7 inches, 13 were pure for tallness while the other 17 gave short and tall plants. Simple counts of talls and shorts in the 17 families gave a total of 1292 shorts and 3807 talls which is a very good 1 : 3 ratio. The average heights of the pure talls varied from 44 to 50 inches. In the splitting families the average height of the tall group varied from 43 to 49 inches and that of the short group from 27 to 30 inches.

TABLE II.

Details of measurements of F_4s of T. 24×T. 282.

F_3 family number	Mean height of F_3 (inches)	F_4 selections	Pure talls mean height, (inches)	Splitting into shorts and talls		Ratios of shorts to talls	
				Shorts	Talls		
				Mean height (inches)	Mean height (inches)	Shorts	Talls
4883	61·5	5293	63	..	..	..	..
..	..	5294	59	..	..	..	..
..	..	5295	60	..	..	..	..
..	..	5296	62	..	..	..	..
..	..	5297	62	..	..	..	..
..	..	5298	60	..	..	..	..
4886	48·7	5300	47	..	..	..	..
..	..	5301	48	..	..	..	..
..	..	5303	50	..	..	..	..
..	..	5304	46	..	..	..	..
..	..	5305	45	..	..	..	..
..	..	5309	44	..	..	..	..
..	..	5319	47	..	..	..	..
..	..	5321	45	..	..	..	..
..	..	5323	48	..	..	..	..
..	..	5324	46	..	..	..	..
..	..	5326	44	..	..	..	..
..	..	5327	44	..	..	..	..
..	..	5328	46	..	..	..	..
..	..	5299	..	27	43	86	245
..	..	5302	..	27	45	93	253
..	..	5306	..	29	48	80	206
..	..	5307	..	28	46	92	257
..	..	5308	..	29	45	81	207
..	..	5310	..	27	45	60	230
..	..	5311	..	28	45	84	211
..	..	5312	..	27	45	77	212
..	..	5313	..	30	49	58	231
..	..	5314	..	27	45	69	220
..	..	5315	..	30	49	64	222
..	..	5316	..	28	46	77	259
..	..	5317	..	31	49	85	247
..	..	5318	..	28	46	81	233
..	..	5320	..	29	46	63	194
..	..	5322	..	30	47	80	180
..	..	5325	..	27	48	62	200
			Total ratios of shorts to talls			1,292	3,807
			Calculated 1 : 3			*1,275*	*3,824*

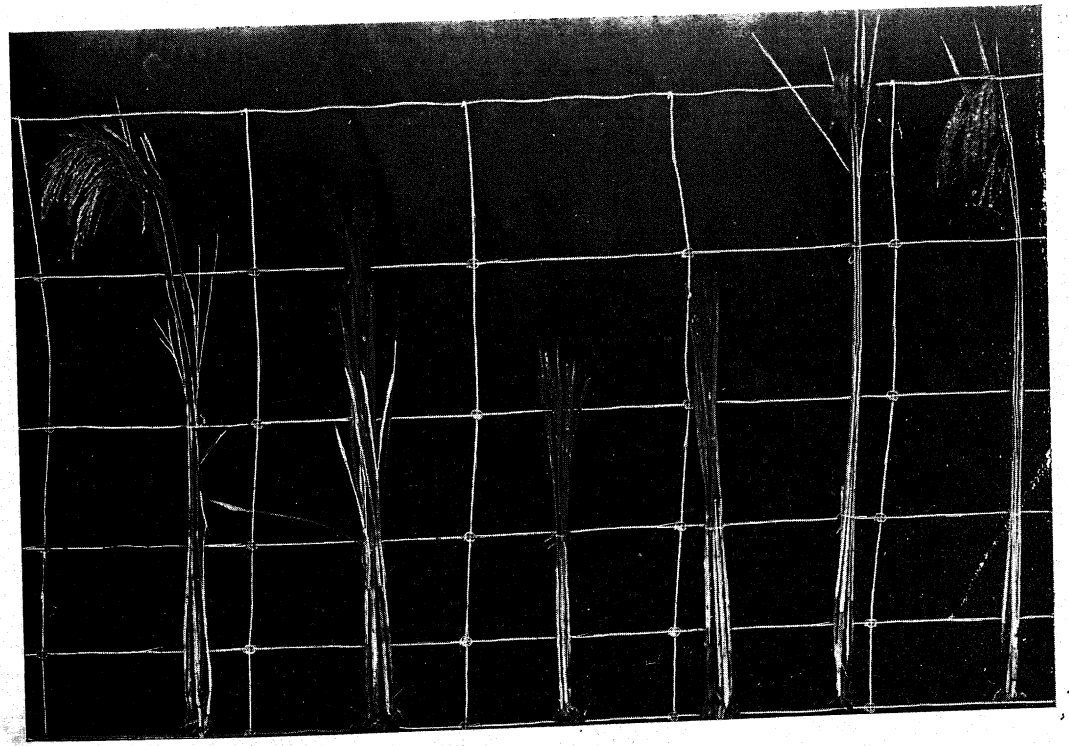
Table II gives the details of the height measurements and the segregating ratios in F_4. Although the simple nature of the shortness to tallness is established, the obtaining of pure breeding talls and shorts with varying mean heights beyond the parental means indicate, that besides the main factor there may be minor factors influencing height. Only a few families corresponding to the parental characters are recovered in the F_3, the majority of them differing from them (Plate XXIX). The case is analogous to the height inheritance studies conducted in beans (Emerson). While the indeterminate and determinate growth types were found to be a simple pair of Mendelian allelomorphs, various pure breeding types of bush and tall beans with intermediate mean heights were obtained which he tried to explain on the hypothesis, that besides the main factor responsible for the pole and bush beans, there were two other minor factors influencing height. The assumption of two such factors would probably explain the inheritance of height in this case as well.

2. Cross T. 24×T. 280.

This particular cross was dealt with in detail in an earlier paper [Ramiah, 1933,] in connection with the inheritance of flowering duration. The mean heights of the parents and the F_1s in the three sets of crosses are given below :—

	Mean height in inches
T. 24/*a*	46
T. 280/*a*	60
F_1	58
T. 24/*b*	45
T. 280/*b*	59
F_1	60
T. 24/*c*	45
T. 280/*c*	59
F_1	59

In everyone of these crosses, the mean height of the F_1 is almost the same as the tall parent indicating that the factors for tallness are predominating in this cross.



Short types.

Fig. 1.—Extracted types from the cross T. 24×T. 282.

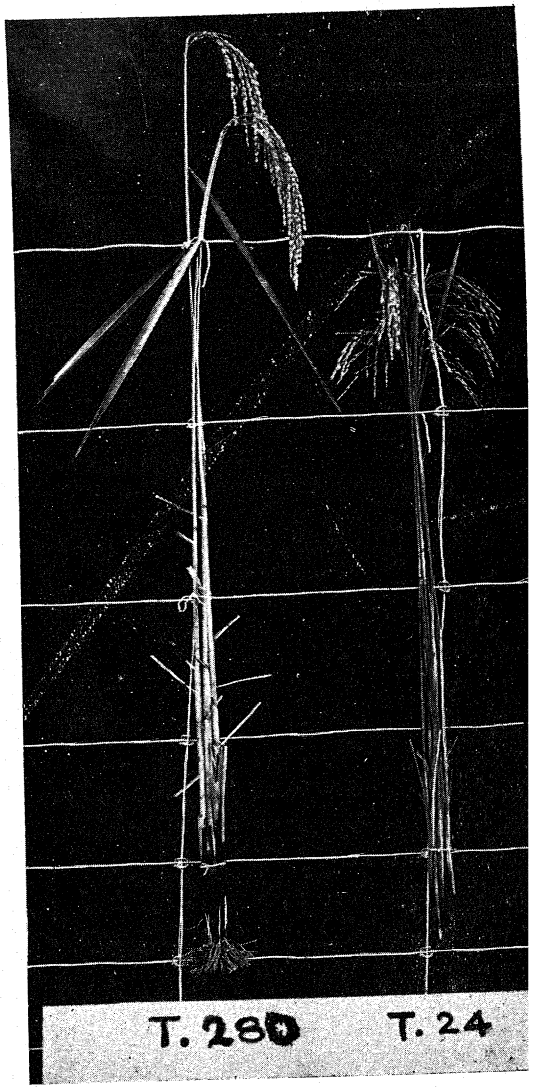


Fig. 2.

TABLE III.

Height frequencies of the F_2s of T. 24×T. 280.

F_1 No.	F_1 height (inches)	F_2 Nos.	Parents	Height frequencies of F_2s (inches)														Mean height (inches)
				13-16	17-20	21-24	25-28	29-32	33-36	37-40	41-44	45-48	49-52	53-56	57-60	61-64	65-68	69-72	73-76	
2107	60	2612	..	..	..	8	13	18	39	121	287	329	235	74	18	6	1	..	..	46·5
..	..	2613	..	..	6	12	7	14	30	71	185	309	307	149	42	6	..	1	..	48·5
..	..	..	T. 280	..	..	..	..	..	..	..	..	..	..	8	15	..	..	..	..	57·3
..	..	..	T. 24	..	..	..	..	..	..	8	12	..	..	..	..	..	..	..	..	41·2
2108	58·5	2616	..	..	..	4	11	15	27	45	137	264	334	228	85	17	3	..	..	50·2
..	..	2617	..	..	1	..	3	6	10	25	70	186	297	240	122	39	6	..	..	52·5
..	..	..	T. 280	..	..	..	..	..	..	..	..	..	..	17	3	..	..	..	..	56·0
..	..	..	T. 24	..	..	..	..	..	..	8	12	..	..	..	..	..	..	..	..	41·2
2106	58·0	2723	..	..	..	..	..	2	2	21	70	174	314	336	200	85	10	1	..	54·0
..	..	..	T. 280	..	..	..	..	..	..	..	..	..	..	1	10	11	1	..	..	61·0
..	..	..	T. 24	..	..	..	..	..	..	..	1	12	14	..	..	..	..	..	..	48·7
2108	58·5	2724	..	..	..	..	2	4	6	14	41	96	192	271	215	124	35	2	1	55·8
..	..	..	T. 280	..	..	..	..	..	..	..	..	..	..	3	14	7	..	..	..	60·0
..	..	..	T. 24	..	..	..	..	..	..	..	1	12	14	..	..	..	..	..	..	48·7

Table III gives the details about the height frequencies of a few typical F_2 families from the above cross. In every case the variation is transgressive (Fig. 4). The ranges of the two parents are quite distinct in that they do not overlap nor even touch each other. There is no indication of any break in the height frequencies of the F_2, indicating that the number of factors concerned must be fairly large. The means of the F_2s are about intermediate between the parents, slightly inclined towards the early parent. That the heights of the F_1s are similar to those of the tall parent and those of the F_2s are intermediate may perhaps be partly accounted for by heterosis which can have the maximum effect only in the F_1. Unfortunately no individual measurement of plant height was done in F_3 selections. Of the several selections that were carried on up to F_6 or F_7 for other studies, types have been obtained with varying mean heights, some with even a shorter height than the short parent, some with intermediate height, and others as tall or even taller than the tall parent. The results would seem to indicate that plant height in this cross was controlled by several factors. The case of another cross where also transgressive variation for height of plants was evident in F_2 may be mentioned. Plate XXX, Fig. 1 gives the two parents involved in this cross and the two extracted types with heights different from those of the parents.

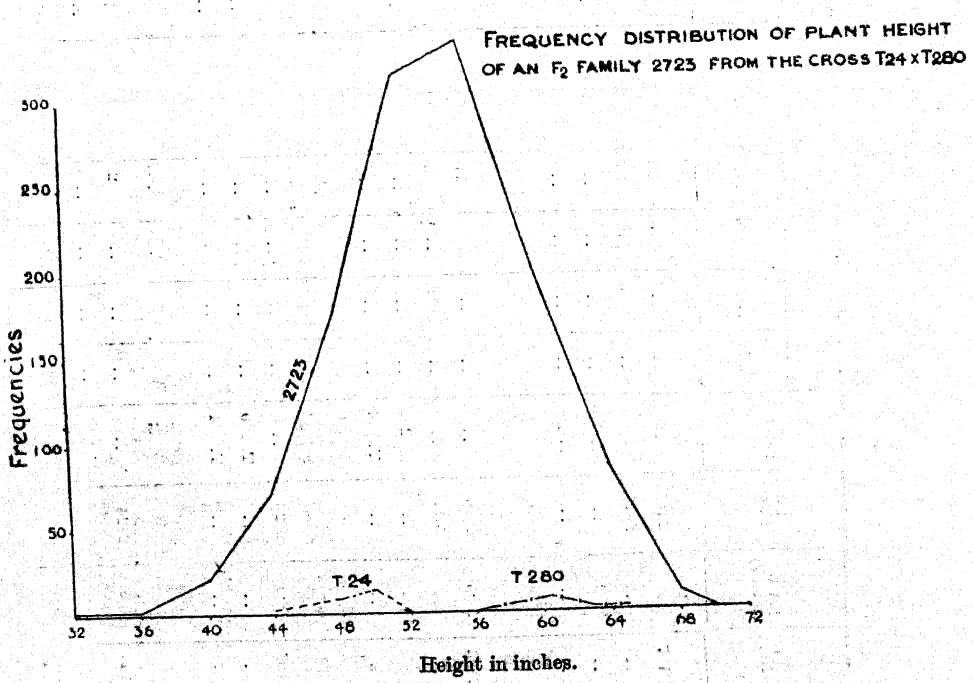
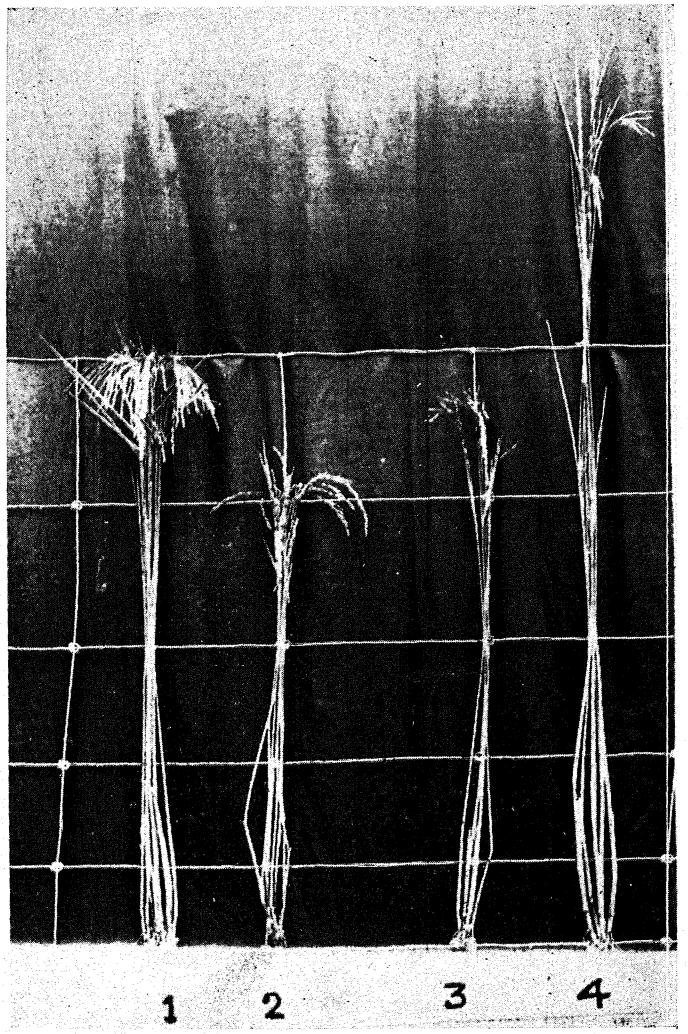


Fig. 4.



Parents. Extracted types.

Fig. 1.

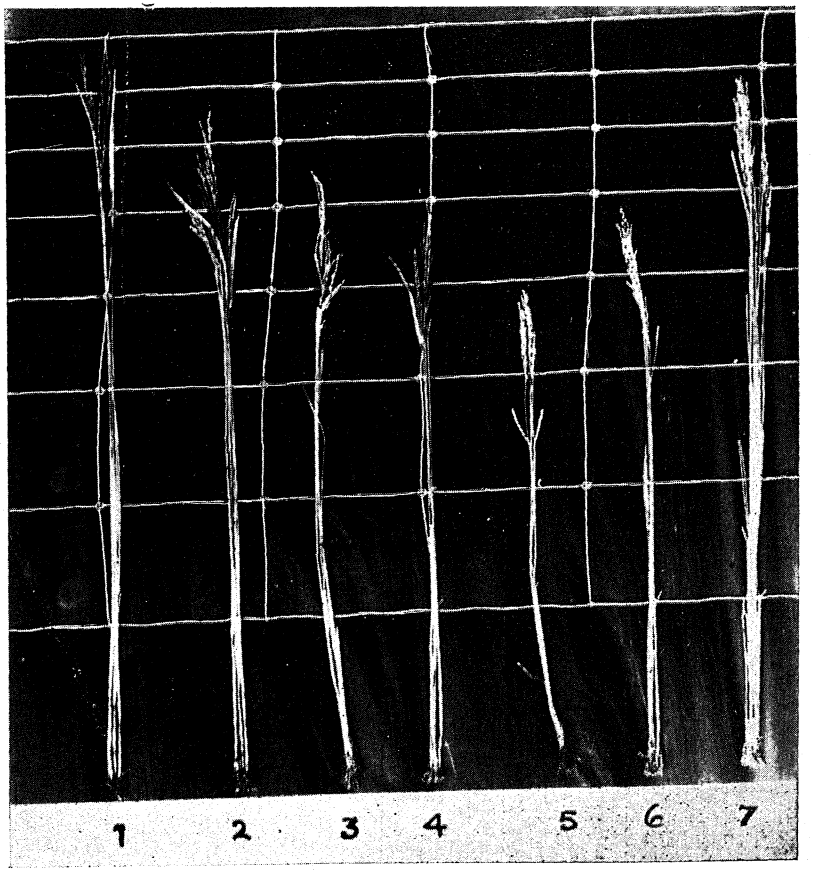


Fig. 2.

(*1*) and (*2*) Extracted tall late and short early types from T. 29×T. 102. (*3*) T. 29. (*4*) T. 102. (*5*) T. 6, (*6*) and (*7*) Extracted short early and tall late types from T. 102×T. 6.

3. Cross T. 24×T. 310.

The inheritance of flowering duration in this cross was discussed earlier [Ramiah, 1933, 1]. The average heights of the two parents over a series of years have been 43 inches and 37 inches respectively. The heights of the F_1, F_2s and F_3s were not noted. In some of the F_4 selections the definite split for height was easily seen. The height was correlated with the flowering duration in that the shorter plants were earlier and the taller plants later. The segregation was very definite. The 3 : 1 ratio of earlies to lates obtained in this group (namely 1,190 earlies : 457 lates) applies to the height segregation as well. In three F_4 families where the 3 : 1 ratios were obtained, the two groups, talls and shorts, were definite without any overlap, with a clear difference of about 8 inches between the mean heights of the two groups. In another F_4 family, 5282, there was no apparent segregation for the character, the frequencies giving a continuous range of height from 36 to 56 inches, exhibiting transgressive variation.

TABLE IV.

Height measurements of F_4s of T. 24×T. 310.

F_4 Nos.	Range of height (inches)	Splitting into shorts and talls				Ratio of short and tall in the definitely splitting families		Pure F_5 types from F_4 with their mean heights	
		Short group		Tall group					
		Range in.	Mean height in.	Range in.	Mean height in.	Shorts	Talls	Shorts	Talls
5282	36-56								
5283		42-46	43	50-54	52	130	39		
5284		40-44	42	46-50	48	121	49		
5285		40-44	42	46-50	48	121	54		
5772								42	
5778								43	
5776									51
5784									50

In the families that were giving the simple split, further selections were made and these confirmed once again the 3 : 1 ratio of shorts to talls. Pure lines of shorts and talls have been extracted from these families with mean heights of 42 and 52 inches respectively. The former corresponds to the average height of the tall parent and the latter is a new type much taller than the same. As in the case of the flowering duration the height segregation can be explained with the help of two factors. If it is assumed that H_1 and H_2 are two factors for short height, domi-

nant to h_1 and h_2 and if each of the parents had one of these factors, the results are easily explained. If the two parents T. 24 and T. 310 are taken to be $H_1H_1h_2h_2$ and $h_1h_1H_2H_2$ respectively we should get a transgressive variation in the F_2. Plants without either H_1 or H_2 will be taller than both the parents and plants with both H_1 and H_2 will be shorter than both the parents. Family number 5282 was in all probability of the $H_1h_1H_2h_2$ composition. If the other three families that give a simple split of shorts and talls are taken to be $H_1h_1h_2h_2$ in composition, they should give 3 $H_1H_1h_2h_2$ to one $h_1h_1h_2h_2$. The $H_1H_1h_2h_2$ class corresponds to the parent T. 24, the slightly taller of the two parents. The class $h_1h_1h_2h_2$ is a new one which on account of the absence of both H_1 and H_2 is taller than T. 24, $H_1H_1h_2h_2$.

4. Cross T. 29×T. 102.

This and the next cross, T. 6×T. 102, have already been dealt with in great detail in connection with the inheritance of flowering duration [Ramiah, 1933, 1]. In both these crosses there was a very definite positive correlation between plant height and flowering duration. This correlation is dealt with in a subsequent article [Ramiah, 1933, 2]. The average height of the two parents over a series of years have been noted as 47 and 48 inches respectively. The height of the F_1 had not been noted, nor was that of the F_2 plants except a mention that the heights of the plants were varying in F_2. Some of the F_3 selections were markedly splitting for height. In two such families the heights of plants were marked individually. The height frequencies of these two families are given in Table V, and have been plotted in Fig. 5.

TABLE V.

Height frequencies of 2 F_3 families in T. 29×T. 102.

F_3 family number	Height frequencies (inches)														Ratio of shorts to talls	
	42	44	46	48	50	52	54	56	58	60	62	64	66	68	Shorts	Talls
2496	1	3	4	14	22	17	15	4	10	7	11	..	1	..	79	30
2509	1	..	2	2	2	10	9	..	..	2	3	2	1	1	26	9
Parents	..	..	..	..	..	..	..	..	..	..	..	..	..	..	105	39

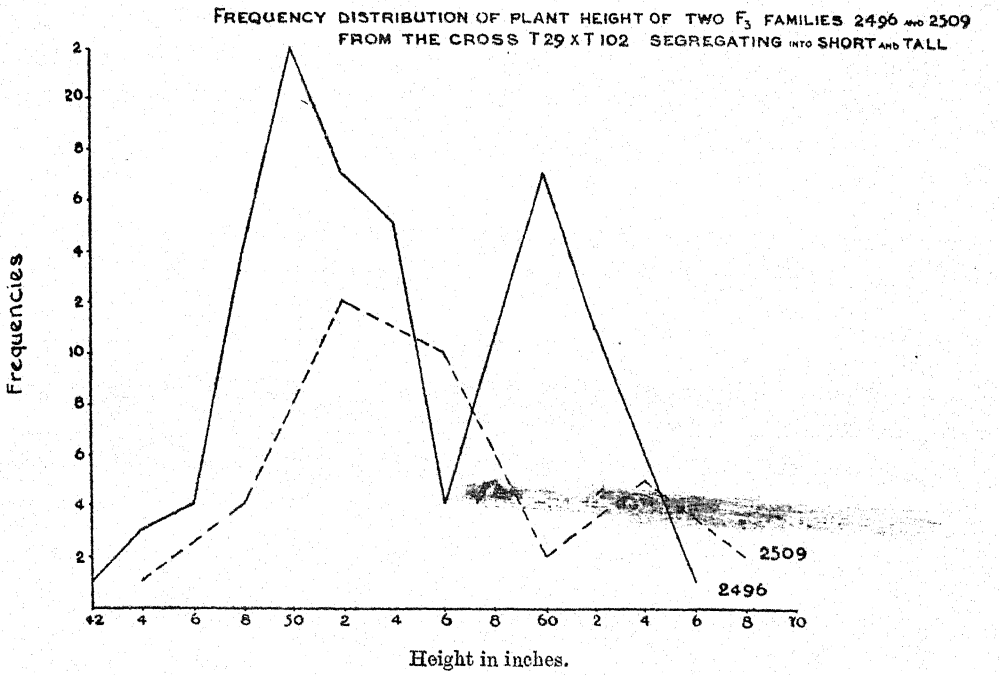


Fig. 5.

In both the families the ranges start from just below the mean height of the two parents and there is a very marked increase beyond the *plus* side of the parental heights. Both the families exhibit a minimum frequency class or a break in the middle at 56 inches, and the totals of the frequencies on either side of this minimum point give a clear ratio of 3 : 1, shorts to talls. The mean heights of the two original parents that had been grown along with the F_4s were T. 29, 45·5 in. and T. 102, 47·0 in. A large number of selections were made in each of the two families, 2496 and 2509 covering the whole range of heights.

2496 group.—Out of the 51 selections in this family with heights on the *minus* side of the minimum frequency class, 17 were pure for short height, while 33 split and gave shorts and talls and one was pure tall. Evidently there has been a mistake about the measurements regarding this last family breeding pure for tallness. The 19 selections taken to the right of the minimum frequency class all bred true for tallness. The mean heights of the pure shorts were not all the same. They varied from 44·5 to 50·5 inches. This variation might be simply due to the different earlies being different in their genetic constitution as was explained in the case of flowering duration. As will be seen in Table VI giving the full details

about this group and that of 2509, the selections at the left extreme of the range bred pure for shortness indicating that the heterozygous group has a greater mean height than the homozygous shorts. The mean heights of the splitting families are all above 50 in. and the mean heights of the pure talls varied from 57 to 63 inches.

TABLE VI.

Height data in F_4 families of T. 29×T. 102.

Family number	Parental height (inches)	PURE SHORTS		SPLITTING FAMILIES	PURE TALLS	
		Range (inches)	Mean height (inches)	Range (inches)	Range (inches)	Mean height (inches)
2496 Group						
2803	42	42-62	52·5	..	..	..
2804	44	40-50	44·7	..	..	..
2805	46	40-48	44·8	..	..	..
2806	46	46-60	50·2	..	..	..
2807	46	38-48	44·5	..	..	..
2808	48	42-54	47·3	..	..	..
2809	48	42-52	48·0	..	..	..
2811	48	46-60	52·8	..	..	..
2812	48	46-60	47·7	..	..	..
2813	48	44-52	48·1	..	..	..
2814	48	36-50	46·0	..	..	..
2817	48	46-54	50·7	..	..	..
2821	50	46-54	50·2	..	..	..
2830	52	42-54	49·0	..	..	..
2840	52	44-62	53·4	..	..	..
2849	54	36-50	43·5	..	..	..
2853	56	42-58	50·5	..	..	..
2810	48	..	..	38-60	..	..
2815	48	..	..	44-60	..	..
2816	48	..	..	46-60	..	..
2818	50	..	..	44-64	..	..
2819	50	..	..	44-64	..	..
2820	50	..	..	42-62	..	..
2822	50	..	..	40-62	..	..
2823	50	..	..	40-60	..	..
2824	50	..	..	36-60	..	..
2825	50	..	..	32-60	..	..
2826	50	..	..	38-62	..	..
2827	50	..	..	42-66	..	..
2828	50	..	..	36-62	..	..
2829	50	..	..	36-62	..	..
2831	52	..	..	36-62	..	..
2832	52	..	..	36-64	..	..
2833	52	..	..	42-68	..	..
2834	52	..	..	40-64	..	..
2835	52	..	..	36-64	..	..
2836	52	..	..	30-64	..	..

TABLE VI—*contd.*

Family number	Parental height (inches)	PURE SHORTS		SPLITTING FAMILIES	PURE TALLS	
		Range (inches)	Mean height (inches)	Range (inches)	Range (inches)	Mean height (inches)
2496 Group—contd.						
2837	52	..	..	32-64	..	..
2838	52	..	..	32-66	..	..
2839	52	..	..	40-62	..	..
2841	54	..	..	38-68	..	..
2842	54	..	..	42-66	..	..
2843	54	..	..	38-66	..	..
2844	54	..	..	38-62	..	..
2845	54	..	..	36-62	..	..
2847	54	..	..	40-64	..	..
2848	54	..	..	34-58	..	..
2850	54	..	..	42-64	..	..
2851	56	..	..	32-62	..	..
2852	56	..	..	34-64	..	..
2846	54	..	..	..	52-64	57·7
2854	58	..	..	..	52-66	59·7
2855	58	..	..	..	50-66	57.2
2856	58	..	..	..	50-66	58·5
2857	58	..	..	..	50-66	60·0
2858	58	..	..	..	52-68	61·5
2859	60	..	..	..	54-66	61·2
2860	60	..	..	..	52-68	60·7
2861	60	..	..	..	52-66	59·7
2862	60	..	..	..	48-64	55·2
2863	60	..	..	..	50-68	58·0
2864	62	..	..	..	50-70	61·0
2865	62	..	..	..	54-72	62·7
2866	62	..	..	..	52-68	58·2
2867	62	..	..	..	48-66	59·0
2868	62	..	..	..	52-70	61·8
2869	62	..	..	..	54-68	62·0
2870	62	..	..	..	60-68	63·6
2871	62	..	..	..	50-66	59·0
2872	62	..	..	..	50-68	59·8
2509 Group						
2873	42	40-50	45·0	..	..	..
2874	46	40-50	46·2	..	..	..
2875	46	40-58	44·3	..	..	..
2878	50	42-54	48·3	..	..	..
2882	52	44-52	47·2	..	..	..
2883	52	42-52	48·1	..	..	..
2884	52	46-56	51·4	..	..	..
2886	54	46-64	55·0	..	..	..
2887	54	46-56	51·7	..	..	..
2891	54	46-60	51·4	..	..	..
2893	54	44-62	51·5	..	..	..

TABLE VI—*concld.*

Family number	Parental height (inches)	PURE SHORTS		SPLITTING FAMILIES	PURE TALLS	
		Range (inches)	Mean height (inches)	Range (inches)	Range (inches)	Mean height (inches)
2876	48	..	..	42-60	..	..
2877	50	..	..	40-60	..	..
2879	50	..	..	38-64	..	..
2880	52	..	..	38-64	..	..
2881	52	..	..	38-66	..	..
2885	52	..	..	42-66	..	..
2888	54	..	..	40-68	..	..
2889	54	..	..	40-68	..	..
2890	54	..	..	44-68	..	..
2892	54	..	..	42-66	..	..
2894	60	..	..	..	50-72	61·0
2895	60	..	..	..	54-64	58·0
2896	62	..	..	..	56-68	59·7
2897	62	..	..	..	50-64	57·0
2898	62	..	..	..	50-64	57·3
2899	64	..	..	..	54-68	61·3
2900	64	..	..	..	54-72	63·4
2901	66	..	..	..	54-68	60·3
Total number of families in each group						
2496 Group		17		33	20	
2509 Group		8		13	8	
		25		46	28	

2509 group.—Out of the 21 selections made in the short group 8 have bred pure for shortness and 13 have segregated, while the 9 selections taken in the tall group have all bred pure for tallness.

In both the groups the mean heights of the pure talls varied slightly, and in no case it was less than 57 inches, a height considerably greater than the parental heights. The break in the height frequencies is not very definite in all the families. It was the definite high correlation between the flowering duration and plant height that made the classification very simple. The frequencies of a typical splitting family in each of the two groups are given in Fig. 6.

FREQUENCY DISTRIBUTION OF PLANT HEIGHT IN TWO F_4 FAMILIES. 2816 AND 2879 FROM THE CROSS T 29 X T 102

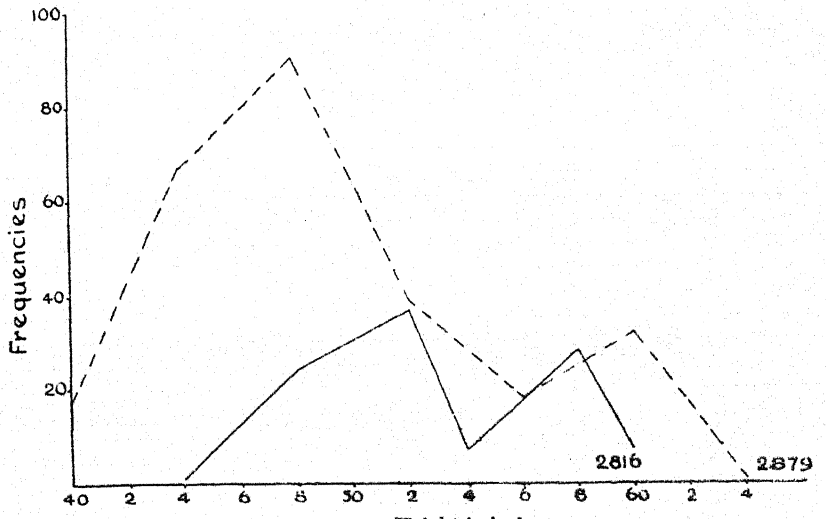


Fig. 6.

The correlation between the flowering duration and plant height may be purely physiological, the same Mendelian factors governing both the characters or it may be that the factors are different, but are present linked in one group. The latter hypothesis appears to be the more probable, because this correlation was not in evidence in some crosses where there was definite segregation for plant height and where there was a considerable amount of variation in the flowering duration as well. For instance in the case of cross T. 24×T. 280 dealt with earlier in connection with the height inheritance, there was a difference of over three weeks between the earliest and the latest plant in the F_2, but still it was not in any way correlated with the height of plant which behaved as a simple split. In fact there was even a small negative correlation between the plant height and flowering duration.

5. Cross T. 6.×T. 102.

This cross continuous with the previous one also proved interesting from the point of view of height inheritance. The mean heights of the parents of this cross when grown along with the F_3s were T. 6, 44·1 in. and T. 102, 48·9 in. *i.e.*, the parent with a longer flowering duration has a greater mean height. The heights of the F_1 and F_2s were not noted. Some of the F_3 families exhibited a definite segregation of shorts and talls. In all the splitting families the height frequencies when plotted exhibited a definite break in the middle, the total shorts to total talls being in the ratio of 1 : 3, just the converse of the previous cross. The frequencies of two typical F_3, one F_4 and 12 F_5 families are given in Table VII.

TABLE VII.

Height frequencies of F_3s, F_4s and F_5s of T. 6×T. 102.

Generation	Number	Character	Height frequencies (inches)																							Parental height (inches)	Mean height of the selections (inches)
			28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62	64	66	68	70			
F_3	2705	Pure short	..	..	..	..	..	..	1	14	13	7	1	3	..	..	..	..	..	..	..	..	..	..		..	..
,,	2701	Split for height	..	..	..	..	..	..	1	1	10	11	10	9	9	17	23	34	23	29	18	3	1	1		..	..
,,	2708	,,	..	1	..	2	14	15	22	20	5	13	29	33	54	43	22	13	5	1	..	..	..	..		..	..
,,	2702	Pure tall	..	..	..	..	..	..	..	..	..	..	2	6	16	33	36	27	20	5	..	..	..	..		..	..
F_4	3381	Split for height	..	..	..	..	..	..	2	9	28	22	32	12	11	35	53	75	85	58	28	9	5	..		..	..
F_5	4169	Pure short	..	..	..	..	8	7	16	39	35	32	10	3	2	..	..	..	..	..	..	..	..	..		44·0	44·7
	4170	,, ,,	..	..	..	..	..	..	2	5	19	46	48	30	30	10	..	..	..	..	..	..	..	..		48·0	48·2
	4171	,, ,,	..	..	..	..	..	1	6	44	105	71	19	3	..	..	..	..	..	..	..	..	..	..		46·0	44·5
	4172	,, ,,	..	..	..	..	4	23	78	69	22	1	2	..	..	..	..	..	..	..	..	..	..	..		46·0	41·0
	4173	,, tall	..	..	..	..	..	..	..	..	..	..	..	3	10	24	41	59	65	32	9	..	..	..		60·0	50·0
	4175	,, ,,	..	..	..	..	..	..	..	..	..	..	5	4	27	43	49	34	39	9	4	..	..	..		64·0	53·2
	4174	Split for height	..	..	..	..	3	21	30	25	15	6	12	32	52	74	51	23	5	..	..	..	..	..		58·0	50·7
	4176	,,	..	..	..	1	3	17	22	16	8	8	25	54	72	51	13	8	4	..	..	..	..	..		60·0	46·0
	4177	,,	..	..	..	..	1	1	6	18	27	21	14	16	46	87	83	69	32	6	1	..	..	..		56·0	45·8
	4178	,,	2	1	4	18	18	14	9	9	12	23	56	40	34	24	6	3	..	..	..	..	..	..		66·0	50·1
	4179	,,	..	..	3	12	28	17	11	11	11	18	47	31	28	27	8	..	..	..	..	..	..	..		58·0	58·2
	4180	,,	..	..	..	..	11	7	4	11	7	5	12	19	22	34	15	17	16	5	2	..	..	..		60·0	56·3

Of the 28 F_4s and F_5s studied, 9 were pure shorts, 15 gave both shorts and talls and 4 were pure talls. The frequencies of two F_5 families are plotted in Fig. 7.

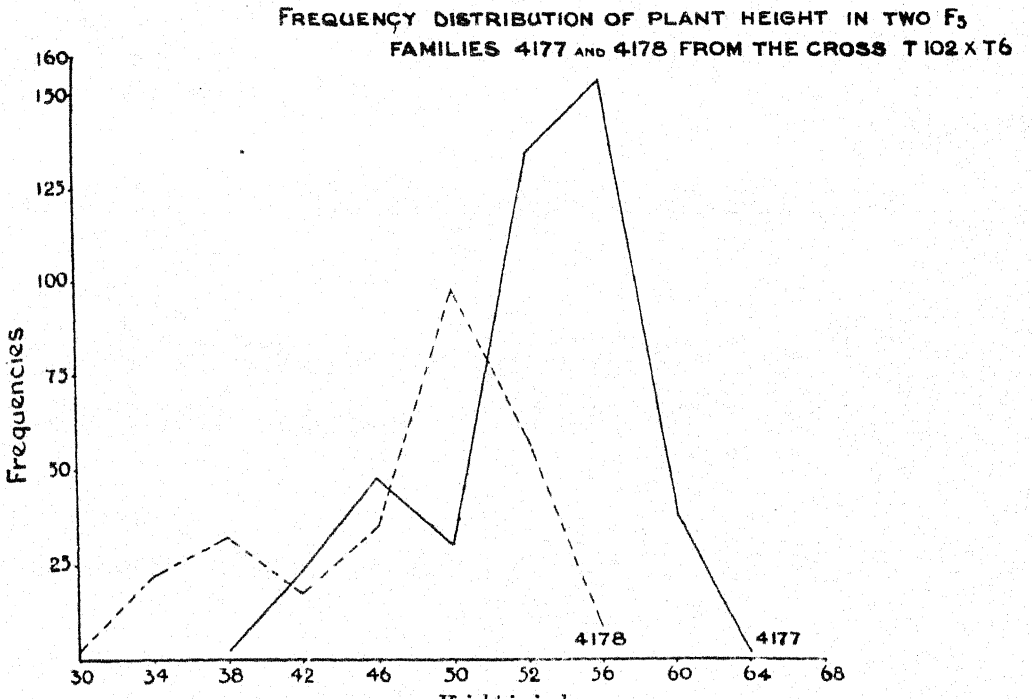


Fig. 7.

Unlike in the previous cross, T. 29×T. 102, it would seem difficult to separate the homozygous from the heterozygous talls from the parental heights. If either the same factors are responsible for both plant height and flowering duration, or if the factors are different but remain linked, the interpretation of inheritance of flowering duration dealt with in an earlier paper [Ramiah, 1933, 1] holds good here. H_1, H_2 and H_3 are three factors controlling height each having a differential effect on the heights of plants. Factors H_1, and H_2 probably have about the same effect and are responsible for a medium height. Factor H_3 is responsible for a taller height than either H_1 or H_2. H_1 is dominant to H_3 and H_3 is dominant to H_2. The three parents according to the above assumptions would be

T. 29	$H_1 H_1 h_2 h_2 H_3 H_3$
T. 102	$h_1 h_1 H_2 H_2 H_3 H_3$
T. 6	$h_1 h_1 h_2 h_2 h_3 h_3$

The cross T. 29 × T. 102 should give, in F_2, 12 shorts to 4 talls, and the cross T. 6×T. 102 should give, in F_2, 12 talls to 4 shorts. This is what was actually obtained. The differential mean height of the pure shorts and pure talls are easily explained as due to their different genetic constitutions.

It is no doubt possible to interpret the results of these two crosses with the help of only two factors, one for tallness and the other an inhibitory factor, but this cannot satisfactorily explain the occurrence of types taller and shorter than either of the parents. The three parents involved in the two sets of crosses and the extracted short early and tall late types from each of the crosses are shown in Plate XXX, Fig. 2.

V. Summary.

The height of plant in rice is a genetic character and can be brought under the purview of Mendelian interpretation. There must be several factors controlling height of plants in rice and varieties should differ in the number and nature of such factors. By crossing varieties, growing the progeny for a number of generations and making selections from them, it would appear possible to get types with new sizes different from the parents. When the number of factors influencing height is large, the interaction among them produces individuals which all conform to a normal distribution. Varieties exhibiting the same mean height may still differ in their composition of genetic factors controlling height. The shortness and tallness may form a simple pair of allelomorphs as in the case of T. 24×T. 282, or may be complicated as in the case of T. 24×T. 280. Shortness may be a simple dominant to tallness or tallness may be a simple dominant to shortness. The behaviour of the F_1s and F_2s even in the complicated cases strictly conform to a Mendelian interpretation on a multiple-factor hypothesis.

There is always a certain amount of variation in the character even among pure lines simply due to the fluctuation of the character resulting from environmental conditions. The parental types got out of crosses giving very simple segregation still exhibit a wider range of variation than the original parents, and they are never identical with the parents with regard to their characters. It cannot be said definitely whether this is simply due to the genetic unstability induced by bringing together individuals of different genetic constitution and ultimate reduction to a homozygous condition, or whether it is due to minor factors which are hypostatic to the main factor and hence not made out easily.

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GENETIC ASSOCIATION BETWEEN FLOWERING DURATION AND PLANT HEIGHT AND THEIR RELATIONSHIP TO OTHER CHARACTERS IN RICE (*ORYZA SATIVA L.*).

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(With one text-figure.)

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I. Association between plant height and flowering duration.

The association existing between these two characters has been established from the studies of the crosses T. 29×T. 102, T. 6×T. 102 and T. 24×T. 310 dealt with in two previous papers [Ramiah, 1933, 1]. That the Mendelian factors responsible for the two characters might be different, but remain linked was also indicated in those studies. The existence of such definite correlation has been brought to light in the progenies of certain other crosses as well. Two examples of such an association are given below. In a cross between T. 1242 and T. 47 done some years ago, there was no apparent segregation for either of the characters in the three F_2 families and the frequencies resembled a normal distribution. There was a difference of nearly 3 feet in plant height between the shortest and the tallest, and there was very nearly a difference of six weeks in the flowering duration between the earliest and the latest. The behaviour of both the characters in the F_2s conformed to the multiple-factor types. The calculated correlation coefficients had a value of about +·7 which was very definite (Table I).

TABLE I.

Correlation table of plant height and flowering duration in an F_2 family, 2574, a cross between T. 1242×T. 47.

(Flowering frequencies—days.)

		83-86	87-90	91-94	95-98	99-102	103-106	107-110	111-114	115-118	119-122	123-126	Total	Coefficient of correlation
Height frequencies (inches)	35-38	4	6	5	4	2	2	..	..	..	..	..	23	+0·65±·01
	39-42	9	26	25	21	18	8	3	..	..	..	..	103	
	43-46	5	35	48	60	54	27	9	2	2	2	..	244	
	47-50	3	12	53	80	91	71	31	13	5	2	..	361	
	51-54	..	2	17	31	75	108	54	47	16	10	4	364	
	55-58	..	1	4	7	15	36	37	36	25	12	1	174	
	59-62	..	..	..	1	2	9	21	39	32	16	3	123	
	63-66	..	..	..	..	..	2	5	12	4	5	..	28	
	67-70	..	..	..	1	1	1	..	..	..	..	..	3	
	Total	21	82	152	205	251	264	160	149	84	47	8	1,423	

As another instance may be mentioned the F_2 family, 604, a natural hybrid, isolated from one of the pure lines. Since the two characters were not studied together individually in this family, the co-efficient of correlation could not be worked out. Fig. 1 represents the frequencies of the two characters plotted separately on the same scale. The figure is a definite bimodal curve exhibiting simple segregation and the heights of plants and the flowering duration are found to vary together proving a definite correlation.

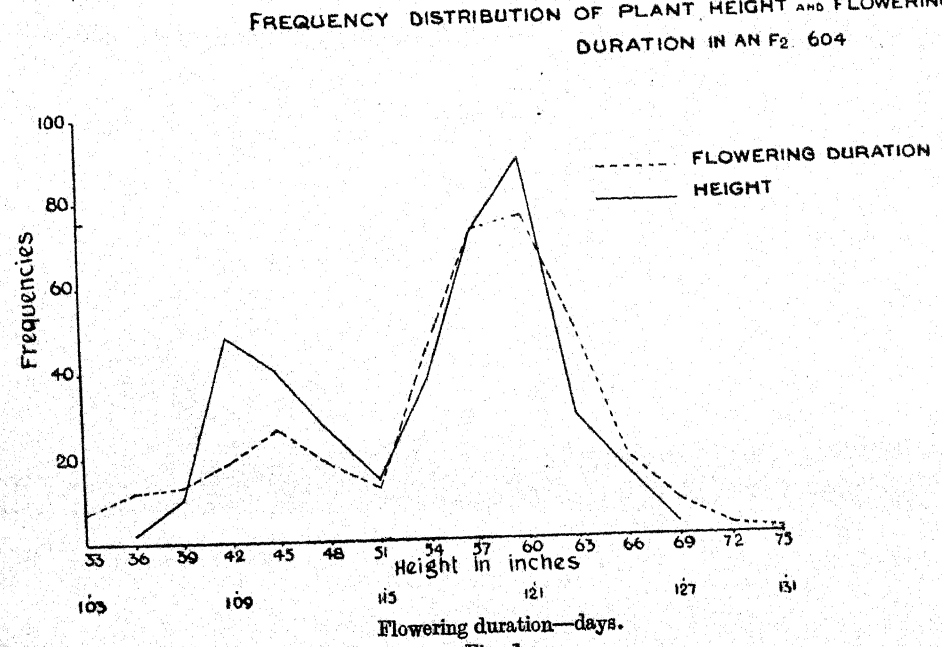

Fig. 1.

The existence of such positive correlation between the two characters is also brought out in the studies of the crosses made among the extracted types out of the two crosses T. 29×T. 102 and T. 6×T. 102. This is dealt with in a separate paper [Ramiah, 1933, 2]. While the correlation is definitely positive in several such independent cases, there are on record instances where the correlation between these two characters is also negative. In the cross T. 24×T. 282 dealt with the inheritance studies of plant height, [Ramiah, 1933, 1] the short parent was later in duration than the tall parent. The F_2 gave a negative co-efficient of $-\cdot18 \pm \cdot01$. Although this correlation is not very definite, there is an indication of the correlation being negative. Although in general the varieties of shorter durations are shorter in stature than varieties of longer duration, the association cannot be said to be absolute. Unless pure lines of different durations and different heights are studied individually for one or two seasons this point cannot be definitely proved. Probably different varieties will differ in such an association according to the Mendelian factors responsible for the characters present in them. It is found that long-duration varieties give a negative correlation between the two characters while early ones give a positive correlation. Why it should be so cannot very well be stated now without further studies bearing on the point. In the cross T. 24×T. 280 which was dealt with in connection with inheritance of flowering duration, two of the F_2s were examined for the association of these two characters. There was an interval of nearly 18 weeks in the flowering durations of the earliest and the latest, and there were a good number of plants that did not flower even after 18 weeks. The correlation co-efficients between plant height and flowering duration in two of these F_2s were Family 2723, $+0\cdot27\pm\cdot02$-Family 2724, $+0\cdot19\pm\cdot02$

For the purpose of calculating these co-efficients only plants that flowered up to the 14th or 15th week after the commencement were taken. From a critical examination of the correlation tables of these families, it was seen that there was an indication of a diagonal dispersion of frequencies from the left hand top quadrant to the right hand bottom one up to the middle, say, for about the first 8 or 9 weeks and there was a definite dispersion from the right side towards the left half. It would appear therefore that the correlation was positive in the early plants and negative in the late plants.

In the crosses, T. 29× T. 102 and T. 6×T. 102, which were giving pure short early types, pure tall late types and also segregating for the two types, the correlation co-efficients were worked out for some of the pure lots which are given below.

From the figures it is seen that the correlation is definitely negative in the pure lates and there is an indication of its being positive in the pure earlies.

	Family number	Correlation co-efficient
Pure tall lates		
	2869	—·293±·042
	2864	—·314±·032
	2866	—·260±·035
Pure short earlies		
	2875	+·102±·066
	2812	+·115±·045

The short early and the tall late parents appear to have different nature of distributions. In the short early parent, the earliest to flower is *tallest* in height and in the tall late parent the earliest to flower is *shortest* in height. This point will be dealt with more fully in a later paper [Ramiah, 1933,2].

An indirect evidence to confirm this observation is obtained from the results recorded in one of the rice sub-stations at Maruteru [Sreenivasan, 1928]. At this station a large number of single plant selections had been made to improve the yields of some of the important local varieties which are all fairly long in duration. The selections of each variety were planted separately with regular spacings and the average plant height and flowering duration of each selection noted. It was found that in all the varieties there was a definite negative correlation between plant height and flowering duration, the co-efficients varying from as low a figure as —0·25 to as high a figure as —0·62 which were all significant.

It therefore appears reasonable to assume that the correlation between height and duration is positive in early lots and negative in late lots. Physiologically also such a phenomenon appears to be reasonable. In an early variety the plants have to rush through their development and naturally the ones that flower first would not have had time enough to reach their maximum height and hence must be shorter in stature than plants that have a slightly longer period for development. In a long duration variety however, there is a sufficiently long interval for the plants to develop properly and hence those that flower earliest should be the most vigorous and show the maximum height, whereas those that flower later may be comparatively weaklings and hence do not attain the same height as the ones that flower earlier. This reasoning fits in with the observations recorded at Maruteru that heavy yielding strains are generally obtained from the more vigorous, taller and earlier flowering plants.

II. Association of plant height and flowering duration with other quantitative characters.

Some of the other quantitative characters that are also associated with plant height and flowering duration are (1) the emergence of the panicle, and (2) the length of the panicle. The height of plants is determined when the plants have put forth all their ears and the ears are nearly ripe. Consequently the length of the ear and its emergence should have an effect on plant height. The inheritance of these two characters, ear length, and ear emergence, have not been worked out completely, but there is enough evidence to indicate that they are Mendelian characters controlled by multiple factors. Two of the F_2 families of the cross T. 24 × T. 280 have been studied in great detail with regard to all these characters. Regarding ear length and ear emergence, T. 280 had a comparatively higher value than T. 24. The F_2 gave a transgressive variation with individuals having both higher and smaller values than the two parents. The co-efficients of correlation worked out independently for all these characters in two F_2 families gave values as below :—

Correlation co-efficients between	Family number 2723	Family number 2724
1. Height and duration	+·27±·019	+·19±·21
2. Height and ear emergence	+·32±·017	+·38±·018
3. Height and length of ear	+·49±·015	+·47±·016
4. Duration and ear emergence	—·46±·016	—·45±·017
5. Duration and length of ear	+·24±·015	+·19±·02
6. Ear emergence and length of ear	+·021±·019	.

All the co-efficients are positive except that between duration and emergence of ear. The co-efficients expressed above are the amounts of total correlations between any two variates. The four variates must have a certain amount of inter-relationship. The partial correlation between any two variates can be determined with the help of the standard formula [Fisher, 1925].

$$r_{12:3} = \frac{r_{12} - r_{13}\, r_{23}}{(1-r^2_{13})\,(1-r^2_{23})}$$

The partial correlation between height and duration has been determined in Family 2723 eliminating the correlations between the other variates (Table II).

TABLE II.

Estimate of partial correlation between height and duration eliminating other variates.

No.	Variates	Correlation co-efficient	Obtained from
1	Height of plant	..	
2	Duration of plant	..	
3	Length of ear	..	
4	Emergence of ear	..	
	r_{12} (total)	+0·27	
	$r_{12\cdot4}$	+0·49	$\frac{r_{12}-(r_{14}\ r_{24})}{(1-r^2_{14})\ (1-r^2_{24})}$
	$r_{13\cdot4}$ eliminating 4	+0·51	$\frac{r_{13}-(r_{14}\ r_{34})}{(1-r^2_{14})\ (1-r^2_{34})}$
	$r_{23\cdot4}$	+0·28	$\frac{r_{23}-(r_{24}\ r_{34})}{(1-r^2_{24})\ (1-r^2_{34})}$
	$r_{12\cdot34}$ (eliminating 3 and 4)	+0·42	$\frac{r_{12\cdot4}-(r_{13\cdot4}\ r_{23\cdot4})}{(1-r^2_{13\cdot4})\ (1-r^2_{23\cdot4})}$

This partial correlation is found to be +·42, a much higher value than +·27 obtained before. It is therefore certain that the correlations found directly between each pair of variates are not the true ones. The low value of the total correlation obtained between height and duration must therefore be due to the interaction of the other variates. The ear emergence directly contributes to the height of the plant and a late plant with potentialities of a greater height might give a lower value of height because of its poor emergence. The correlation between height and duration when the emergence alone is eliminated comes to +·49 a very much higher value than the total correlation, +·27. The correlation between ear length and ear emergence is practically *nil*, but it is quite possible that this may give a significant value when the other variates are eliminated.

The existence of a negative correlation between ear emergence and flowering duration was also evident in the progenies of a natural hybrid from one of the pure lines, T. 100, which was segregating for flowering duration. Some of the F_4 families were measured for ear emergence (which had been done individually for flowering duration) and the co-efficient of correlation was calculated. It gave a value —·76± ·004, a very much higher value than what was obtained in the families of T. 24× T. 280 cross mentioned earlier. In the latter families all the very late plants which did not flower up to the 13th or 14th week from the commencement of flowering were left out and this might have contributed partly to the lower value of the co-efficient. It cannot be said at present whether this relationship between duration and emergence is purely physiological or is due to partial linkage of Mendelian factors responsible for them.

The correlations between height and ear emergence and that between height and length of ear are very definite, while that between duration and length of ear does not seem to be very marked.

III. Association of plant height and flowering duration with morphological characters.

1. Colour of glumes.

Cross T. 47 × T. 1242.—Two of the F_2s of this cross, where the flowering duration was tabulated along with the colour of glumes, showed a partial association of the two characters. The F_2 of another independent, cross, T. 1 × T. 185, where also the same character, glume colour, was involved, gave the same sort of relationship between glume colour and flowering duration. The frequencies of the flowering duration tabulated with glume characters in these F_2 families are given in Table III.

Table III.

Flowering duration tabulated with colour of glume.

Flowering frequencies in days	F_2 of T. 47 × T. 1242				F_2 of T. 1 × T. 185	
	Family 2569		Family 2573		Family 2587	
	Plants with		Plants with		Plants with	
	Brown glume	Gold glume	Brown glume	Gold glume	Brown glume	Gold glume
81-84	2	..	6	..	..	..
85-88	33	5	55	4	..	..
89-92	91	9	126	12	..	..
93-96	118	18	204	31	7	..
97-100	113	31	223	36	61	5
101-104	119	19	170	41	182	31
105-108	66	38	99	51	265	41
109-112	48	54	60	78	194	47
113-116	21	30	31	41	118	48
117-120	7	15	8	15	55	47
121-124	1	3	4	8	43	46
125-128	..	..	..	..	17	15
129-132	..	..	..	..	14	21
133-136	..	..	..	..	13	14
137-140	..	..	..	..	3	4
Totals	619	222	986	325	972	319
Mean duration	99·8 ± ·20	106·6 ± ·36	99·3 ± ·16	106·8 ± ·30	110·0 ± ·16	118·5 ± ·32
Standard deviation	7·4	8·1	7·2	8·2	8·4	8·4

It is definite from the tabulations that the plants with the gold coloured glumes are a little later in duration than those with brown glumes. The means of flowering durations of the two groups, brown glume and gold glume, taken separately are different in every family and the differences are highly significant in terms of the standard error. The test of independence in a $2 \times n$ classification applied to one of the families gives an extremely small value of P less than ·01 proving that the glume colour difference in the classification by different groups of flowering durations is significant [Fisher, 1925]. It had been shown [Parnell, 1922] that the gold colour of glumes is due to an inhibitory factor, **I**, which makes the brown into gold. It is therefore evident that this factor **I** is in some way connected with the factors responsible for duration. Since it has been shown already that the flowering duration is highly correlated with the height in these families it follows that the factor **I** is also connected with the factors for height. The height frequencies tabulated with the glume colour (Table V) is exactly similar to that between flowering duration and glume.

TABLE IV.

Test of independence in a 2×n classification in Family 2573.

Glume	Flowering frequencies in days						
	92 and below	93-98	99-104	105-110	111-116	117 and above	Total
Brown	187	317	280	129	61	12	986
Gold	16	49	59	92	78	31	325
Totals	203	366	339	221	139	43	1,311
	*9·97	8·17	3·17	10·77	23·45	16·57	72·10

* In millions.

$$\lambda^2 = \frac{72{\cdot}10 \text{ millions}}{986 \times 325} = 225.$$

For $n = 5$ and $\lambda^2 = 225$, the value of $P = {\cdot}000000$.

TABLE V.

Height frequencies tabulated with glume colour in the F_2s of T. 47 × T. 1242.

Height frequencies (inches)	FAMILY 2569		FAMILY 2573	
	Brown glume	Gold glume	Brown glume	Gold glume
34	—	—	3	—
36	3	1	7	1
38	5	1	25	—
40	18	2	44	1
42	53	3	117	12
44	77	5	99	4
46	82	3	130	24
48	133	17	176	27
50	92	22	125	26
52	63	35	85	33
54	36	26	79	34
56	25	19	57	44
58	21	28	15	35
60	8	34	15	38
62	3	13	4	29
64	—	7	3	8
66	—	5	—	7
68	—	1	2	2
70	—	1	—	—
72	—	—	—	1
Total	619	222	986	325
Mean height	48·3 ± ·19	54·8 ± ·17	47·9 ± ·17	54·5 ± ·34
Standard deviation	4·69	5·74	5·29	6·10

2. Colour of rice.

Parnell [1922] had shown that the factor **I** inhibits the gold and changes any form of gold in the glumes to a corresponding form of brown and that this factor is necessary for the production of red rice by the factor **R**. In the absence of **I**, the factor **R** produces golden rice. He had given the ratios of splittings for rice colour in the two families 2569 and 2573 showing segregation for factor **I** in the presence of **R**. In all the cases it was shown that the red rice of the brown-glumed group had been replaced by golden rice in the gold-glumed group. It is therefore evident that the factor **R** is also connected with the duration factors and hence also with the height factors.

3. Nature of straw, lodging or non-lodging.

Recently the inheritance of the lodging nature of the straw has been under investigation at the Paddy Station, Coimbatore, in a certain number of crosses specially made for the purpose. While these results would be discussed in a

separate publication, it may be pointed out here that there has been found a definite association between the nature of the straw and the flowering duration of the plant. Where there has been a segregation for both the characters, nature of straw and flowering duration, it is found that plants with early duration tend to lodge while most of the plants with non-lodging or erect habit are later in duration. Though the plant height was also varying in these families no apparent relationship was noticed either between height and duration or between height and nature of straw.

4. Yield of plants.

Lastly we come to the most important association, that of yield of plants with height and duration. It has been shown [Ramiah 1933, 1] that the F_3 progenies of the cross, T.29 × T.102, were giving a simple 3 : 1 ratio of short early to tall late. In 7 of the F_4 families when the plants were ready for harvest, the two groups of plants, short early and tall late, were harvested separately and the bulk yields determined per group. The results of such weighments are given in Table VI.

TABLE VI.

Yields of Short early and Tall late plants harvested separately in F_4 families of T. 29 × T. 102.

Family number	Groups	Number of plants	Yield in grams.		Percentage increase
			Total	Per plant	
2879	Short early	124	1,080	8·71	233
	Tall late	32	650	20·31	
2880	Short early	258	2,230	8·64	362
	Tall late	100	3,130	31·30	
2881	Short early	315	2,280	7·24	458
	Tall late	94	3,130	33·20	
2885	Short early	77	810	10·52	377
	Tall late	29	1,150	39·66	
2886	Short early	85	960	10·11	460
	Tall late	23	1,070	46·52	
2889	Short early	101	900	9·00	273
	Tall late	44	1,080	24·55	
2890	Short early	72	920	12	291
	Tall late	25	930	37·20	
Total for all 7 families	Short early	1,032	9,180	9·57	348
	Tall late	347	11,140	33·25	

It is found that the yields of the tall late plants are considerably higher than those of the short earlies, the increase varying from 233 to 460 per cent. in the different families. Unfortunately the border rows of plots which usually give a very much bigger yield than the middle rows were not eliminated, nor the yields of plants recorded individually according to their duration. But it is definite that

the enormous increase of nearly 350 per cent., the average of the 7 families, must be largely associated with the duration.

In the following year, the same kind of separation of plants into short earlies and tall lates was done in the case of 3 F_5 families resulting from the cross 6 × T. 102 which was giving a 1 : 3 ratio of short early to tall late. The separation was done in greater detail, row by row, and according to the duration of the plants and in every case rejecting the border rows (Table VII).

TABLE VII.

Yields of plants—Short early and Tall late—according to their duration in F_5 families of T. 6 × T. 102.

Flowering duration (days)	Family 4174			Family 4176			Family 4178		
	Number of plants	Yield		Number of plants	Yield		Number of plants	Yield	
		Total	Per plant		Total	Per plant		Total	Per plant
Short early									
78	1	19	19		—	—	—	—	—
80	9	152	16·9	10	133	13·3	2	33	16·5
82	9	173	19·2	12	143	11·9	11	125	11·4
84	9	193	21·4	8	82	10·3	10	130	13·0
86	14	263	18·8	4	65	16·3	2	23	11 5
88	11	205	18·6	4	51	12·8	7	87	12·4
90	6	95	15·8	1	7	7·0	9	124	13·8
92	6	90	15·0	1	9	9·0	2	24	12·0
94	4	64	16·0	2	28	14·0	3	46	15·3
96	—	—	—	1	7	7·0	—	—	—
Total	69	1,254	18·1	43	525	12·0	46	592	12·9
Tall late									
104	—	—	—	10	273	27·3	—	—	—
106	3	144	48·0	28	786	28·1	—	—	—
108	10	410	41·0	54	1,468	27·2	19	517	27·2
110	27	713	34·0	32	790	24·7	37	875	23·7
112	42	1,345	32·0	18	442	24·6	24	522	21·7
114	60	1,920	32·0	13	249	19·2	35	782	22·3
116	29	860	29·7	2	16	8·0	13	228	18·3
118	10	192	19·2	—	—	—	2	31	15·5
Total	175	5,584	31·9	157	4,024	25·6	130	2,965	22·8
Percentage increase in yield of tall late over short early		177			214			177	

The percentage increase of yield in the tall late plants over the short early ones is 177 in Family 4174, 214 in 4176 and 177 in 4178, with an average of 182

per cent. for the three families together. The yield per plant in the three families according to its duration is interesting. In the early group the variation of yield with the duration is not very regular. The maximum yield is probably obtained in a plant with a flowering duration somewhere in the middle. In the late group, however, the maximum yield is invariably obtained in plants flowering earliest. There is a definite tendency observed in all the three families for the yield per plant to fall down as the duration increases and the plants flowering latest are the lowest in yield. This fits in with the explanation given earlier for obtaining a definite negative correlation between height and duration in the late families. The earliest, being the most vigorous in the late groups, yield also the highest. The yields not varying regularly with the duration in the early group probably accounts for the rather low positive correlation obtained between height and duration. The mean difference in duration between the early and the late group was about three weeks.

A similar analysis of yields in another group of families which was also giving a simple 3 : 1 ratio of short early to tall late and where the difference in duration between the two groups was under 2 weeks also gave a significant increase in yield in favour of the tall late plants, though the increase was not as high as in the cases dealt above. The group referred to is from the cross T. 24 × T. 310, dealt with in connection with the inheritance studies of height and duration. In some of the F_4 families of this cross which were splitting into short early and tall late, the two groups of plants were harvested separately and the yield per plant determined. Only 3 rows of plants had been planted in each family and as the marginal effect was common to the two end rows, the central row was left out keeping only the two outside rows for yield records. The results are given in Table VIII.

TABLE VIII.

Yield per plant in the two groups, short early and tall late, in the cross, T. 24×T. 310.

Generation	Number	Character	Number of plants	Yield in grams		Percentage increase
				Total	Per plant	
F_4	5283	Short early	81	2,941	36·3	28
		Tall late	24	1,115	46·5	
,,	5284	Short early	71	2,263	31·9	23
		Tall late	33	1,297	39·1	
,,	5285	Short early	68	2,617	38·5	19
		Tall late	34	1,560	45·9	
Total for all 3 families		Short early	220	7,821	35·5	23
		Tall late	91	3,972	43·6	
	5770	Short early	439	15,477	35·3	44
		Tall late	182	9,254	50·8	

In three F_4 families the tall late plants give an increased yield of 23 per cent. over the short early plants. In one of the F_5 families this increase was as much as 44 per cent. The results give an indication that the difference in yield between an early and a late plant in general is proportional to the difference in duration between the two. The bigger the difference in duration the greater is the yield difference likely to be. The above results thus definitely prove that the most important economic character of a plant, namely, its yield, is intimately associated with its duration and hence probably with its stature as well.

V. Summary.

There has been established a very definite and strong association of the two characters, height and flowering duration. The correlation between these two characters is generally positive but in some cases it is also negative. Although very definite proof is still not available on the point, it is considered from the behaviour of the parents in the several crosses dealt with in the paper that the correlation is positive in early varieties and negative in late varieties. Such a behaviour seems to be consistent with a physiological explanation. It is reasonable to expect a physiological correlation between size and duration of growth. Obviously, an extremely early plant cannot in the few weeks of its growth attain a height equal to that ultimately reached by another plant whose period of growth extends over a much longer period. It is quite likely that genetic correlations occur between factors for distinct quantitative characters. These and the pysiological correlations make the results more difficult of interpretation but do not throw them out of the realm of Mendelian phenomena. It is suspected that the correlation between the two characters is necessarily genetic on account of the absence of correlation and segregation for the characters in the progenies of certain crosses whose parents did differ greatly from each other for the characters.

These two characters are also found to be associated with other quantitative characters like the length of the ear, and the emergence of the ear. They are also very definitely associated with the final yield, the later and taller plants giving a very much increased yield over the earlier and shorter plants. The larger the difference in the characters is between the plants, the greater is the yield difference likely to be.

The two characters, height and duration, are also associated with such qualitative characters as the colour of the glume and the colour of rice. An association has also been noted between flowering duration and the lodging or erect nature of the straw.

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INHIBITORY FACTOR HYPOTHESIS AND THE INHERITANCE OF FLOWERING DURATION AND PLANT HEIGHT IN RICE (*ORIZA SATIVA L.*).

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I. INTRODUCTION.

In two previous articles [Ramiah 1933] the inheritance of flowering duration and plant height was discussed in the progenies of two sets of crosses involving three parents. In one set, T. 29×T. 102, there was a simple segregation into short early and tall late plants in a 3 : 1 ratio. In the other set, T. 102×T. 6, there was again a simple segregation of short early and tall late plants in a 1 : 3 ratio, just the converse of the first set. Thus in two crosses, keeping one parent the same in both, short early showed simple dominance over tall late in one, and in the other tall late showed simple dominance. From these two sets of crosses the following four pure types were obtained :—

Short Early Dominant designated S. E. D.
Short Early Recessive „ S. E. R.
Tall Late Dominant „ T. L. D.
Tall Late Recessive „ T. L. R..

The following six sets of crosses were made from among these four extracted types and also the cross between T. 29 and T. 6. The article deals with the

genetic constitution of these extracted types and the results of the crosses are explained both on a multiple-factor hypothesis and on an inhibitory-factor hypothesis.

Cross number

1. S. E. D. (T. 139) × S. E. R. (T. 155)
2. S. E. D. „ × T. L. D. (T. 156)
3. S. E. D. „ × T. L. R. (T. 140)
4. S. E. R. (T. 155) × T. L. D. (T. 156)
5. S. E. R. „ × T. L. R. (T. 140)
6. T. L. D. (T. 156) × T. L. R. (T. 140)

In each cross the parents were grown by the side of their F_1 and F_2 generations giving the same spacing to get comparable records of height and duration. In every cross two F_2s, and in some, four F_2s, each consisting of over 500 plants were grown to get a population big enough for the statistical estimation of the characters. The F_3 families studied in cross 1 were, however, too few and had too small numbers in each, but the F_2 results have been definite enough in every case to support the factorial interpretation adopted.

The S. E. D. and T. L. R. types were derived from the F_4 families of cross T. 29×T. 102. The short early extract was slightly shorter and earlier and the tall late extract was much taller and later than the respective parents. Assuming that height and duration were controlled by the same factors and adopting the interpretation then given, there were two factors for earliness and shortness, $\mathbf{E_1}$ and $\mathbf{E_2}$, and one factor of lateness and tallness, $\mathbf{L_1}$, and $\mathbf{E_1}$ was dominant to $\mathbf{L_1}$ and $\mathbf{L_1}$ was dominant to $\mathbf{E_2}$. The particular F_4 family from which the two types were obtained was explained then to have been of the constitution $\mathbf{L_1L_1E_1e_1E_2e_2}$ giving 12 short earlies to 4 tall lates. Though the short earlies could be either $\mathbf{L_1L_1E_1E_1E_2E_2}$ or $\mathbf{L_1L_1E_1E_1e_2e_2}$, the actual type being shorter and earlier than the parents, its constitution should have been only $\mathbf{L_1L_1E_1E_1E_2E_2}$. The tall late extract from this could be either $\mathbf{L_1L_1e_1e_1E_2E_2}$ or $\mathbf{L_1L_1e_1e_1e_2e_2}$, but the type being taller and later than the parent, its constitution should have been $\mathbf{L_1L_1e_1e_1e_2e_2}$.

The S. E. R. and T. L. D. types were extracted from a F_4 family of the cross T. 6×T. 102. It was shown previously that the particular F_4 family which gave rise to these types should have been of the constitution $\mathbf{L_1l_1e_1e_1e_2e_2}$. The short early extract should therefore be $\mathbf{l_1l_1e_1e_1e_2e_2}$ resembling T. 6, and the tall late extract $\mathbf{L_1L_1e_1e_1e_2e_2}$ taller and later than the parents.

On the inhibitory factor hypothesis **L** denotes the factor for lateness and tallness and factor **I** completely inhibits **L** and makes the plant short early. The factorial composition of the 4 types can then be represented as :—

—	On a 3-factor hypothesis	On the inhibitory-factor hypothesis
1. S. E. D.	$I_1L_1E_1E_1E_2E_2$	LLII
2. S. E. R.	$l_1l_1e_1e_1e_2e_2$	llii
3. T. L. D.	$I_1L_1e_1e_1e_2e_2$	LLii
4. T. L. R.	$L_1L_1e_1e_1e_2e_2$	LLii

According to the multiple-factor hypothesis, there can be any number of Mendelian factors affecting a particular character of the plant, some of which may be dominant and some recessive.

II. Crosses.

(*a*) F_1 *behaviour.*

The characters of the parents and their F_1s are given in Table I.

Table I.

Flowering duration and plant height of F_1s and parents.

Number	Cross		Height (inches)	Flowering duration (days)
1	S. E. D. × S. E. R.	T. 139	40·7	76
		T. 155	40·7	79
		F_1. 6314	41·0	77
2	S. E. D. × T. L. D.	T. 139	42·0	79
		T. 156	52·0	102
		F_1. 6318	42·0	80
3	S. E. D. × T. L. R.	T. 139	40·0	82
		T. 140	56·0	101
		F_1. 6325	41·0	82
4	S. E. R. × T. L. D.	T. 155	40·0	80
		T. 156	52·0	104
		F_1. 6328	52·0	98
5	S. E. R. × T. L. R.	T. 155	38·0	81
		T. 140	53·0	104
		F_1. 6330	51·0	99
6	T. L. D. × T. L. R.	T. 156	64·0	107
		T. 140	59·0	100
		F_1.	64·0	100

It is seen that except in crosses 1 and 6, the parents differ from each other in height and duration but still the F_1s are just like either of the parents without

blending. In crosses 4 and 5 the duration of F_1 is not strictly intermediate but more inclined towards one parent. The close association between the two characters is also manifest. The behaviour of the F_1s would seem to suggest that the number of factors involved cannot be many. The factorial compositions of the F_1s would be :

—	Three-factor hypothesis	Inhibitory-factor hypothesis	Character
1. S. E. D. × S. E. R.	$L_1l_1E_1e_1E_2e_2$	I IIi	Short early
2. S. E. D. × T. L. D.	$L_1L_1E_1e_1E_2e_2$	I LIi	,, ,,
3. S. E. D. × T. L. R.	$L_1L_1E_1e_1E_2e_2$	LLIi	,, ,,
4. S. E. R. × T. L. D.	$L_1l_1e_1e_1e_2e_2$	LIii	Tall late
5. S. E. R. × T. L. R.	$L_1l_1e_1e_1e_2e_2$	LIii	,, ,,
6. T. L. D. × T. L. R.	$L_1L_1e_1e_1e_2e_2$	LLii	,, ,,

(*b*) *F_2 behaviour.*

The F_2s of the crosses were grown under uniform conditions along with the parents. The marking of flowering dates and measurements of heights were done individually in all the F_2 families. During flowering two distinct flushes could be made out and it was quite easy to separate the plants into two groups, early and late, even by eye judgment. The heights were found to go with the duration in every case, the shorter plants flowering earlier than the taller plants. The frequencies for height and flowering duration gave a definite bimodal curve when plotted.

In cross 1, according to the factorial composition of the F_1 indicated, we should get in the F_2s the following phenotypes in the proportion noted against each.

Three-factor hypothesis			Inhibitory-factor hypothesis (two factor)		
Phenotypes	Proportion	Description	Phenotypes	Proportion	Description
$L_1E_1E_2$	27	Short early	..	..	....
$L_1E_1e_2$	9	,, ,,	LI	9	Short early
$L_1e_1E_2$	9	Tall late	lI	3	,, ,,
$l_1E_1E_2$	9	Short early	li	1	,, ,,
$l_1E_1e_2$	3	,, ,,	Li	3	Tall late
$L_1e_1e_2$	3	Tall late	..	..	....
$l_1e_1E_2$	3	Short early	..	..	....
$l_1e_1e_2$	1	,, ,,	..	..	....
Short early to tall late 52 : 12 or 13 : 3			Short early to tall late 13 : 3		

Actually the following numbers were got in the F_2s of this cross.

TABLE II.

F_2 number	Proportion of short early to tall late	
	Short early	Tall late
6552	508	119
6553	543	91
6596	323	104
6591	329	86
Total	1,703	400 $\frac{\text{Dev.}}{\text{S. E.}} = 0{\cdot}33$
Calculated 13 : 3	*1,719*	*394*

The production of tall lates from the apparently short early parents and also the distinct ratios of 13 : 3 obtained suggest the inheritance of multiple factors in the inheritance. Fig. 1 gives the frequencies for height and duration for one of the F_2s.

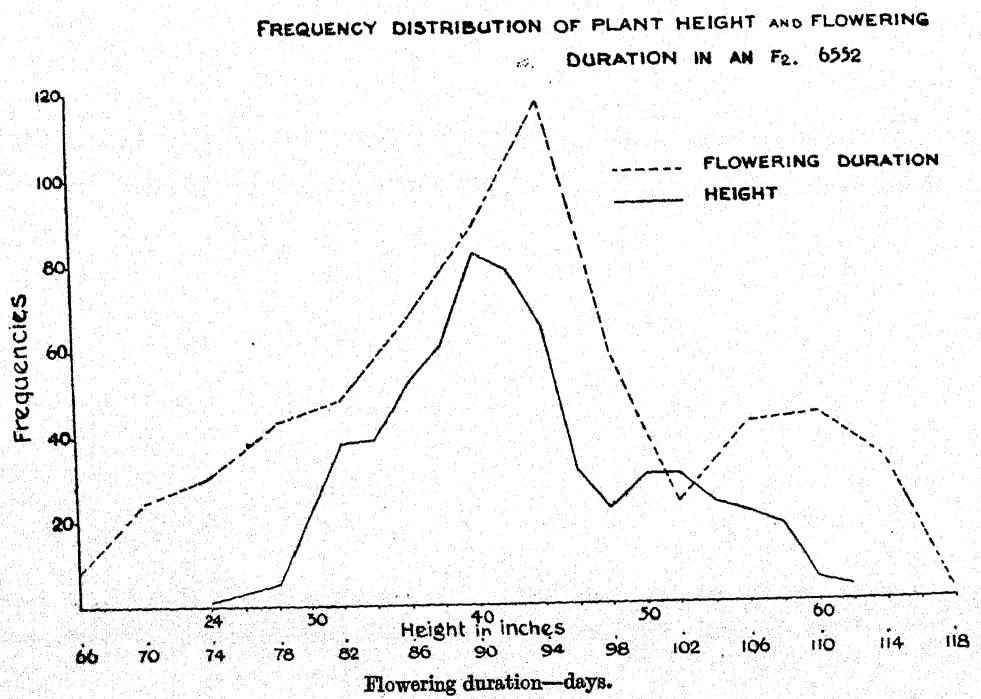

Fig. 1.

In crosses 2 and 3 (F_1—L_1 L_1 E_1 e_1 E_2 e_2 or **LLIi**) the segregation should be as below :—

Three-factor hypothesis		Inhibitory factor hypothesis (two factor)	
Phenotypes	Proportion	Phenotypes	Proportion
L_1 E_1 E_2	9 short early	LI	3 short early
L_1 E_1 e_2	3 „ „	..	
L_1 e_1 E_2	3 tall late	..	
L_1 e_1 e_2	1 „ „	Li	1 tall late
	Short early : tall late=12 : 4.		3 : 1.

The ratio obtained actually conforms to the theoretical expectations. (Table III.)

Table III.

No.	Cross	F_2 No.	Proportion of short early to tall late	
			Short early	Tall late
2	S. E. D.×T. L. D.	6558	448	155
		6559	561	160
		6560	458	138
		6561	513	175
		Total	1,980	628 $\frac{\text{Dev.}}{\text{S. E.}} = 1{\cdot}1$
		Calculated 3 : 1	*1,956*	*652*
3	S. E. D.×T. L. R.	6569	384	125
		6570	462	183
		Total	846	308 $\frac{\text{Dev.}}{\text{S. E.}} = 1{\cdot}3$
		Calculated 3 : 1	*865*	*289*

In crosses 4 and 5 (F_1=L_1 l_1 e_1 e_1 e_2 e_2 or **Llii**), F_1 is tall late and the segregation of phenotypes in F_2 should give 3 of tall late to one of short early and this agrees with the results obtained. (Table IV.)

TABLE IV.

No.	Cross	F_2 No.	Proportion of short early to tall late	
			Short early	Tall late
4	S. E. R. × T. L. D.	6574	146	441
		6575	114	359
		Total	260	800 $\frac{\text{Dev.}}{\text{S. E.}} = 0{\cdot}35$
		Calculated 1 : 3	*265*	*795*
5	S. E. R × T. L. R.	6577	99	286
		6578	100	283
		Total	199	569 $\frac{\text{Dev.}}{\text{S. E.}} = 1{\cdot}0$
		Calculated 1 : 3	*192*	*576*

Fig. 2 represents the frequencies of height and duration of one F_2 family.

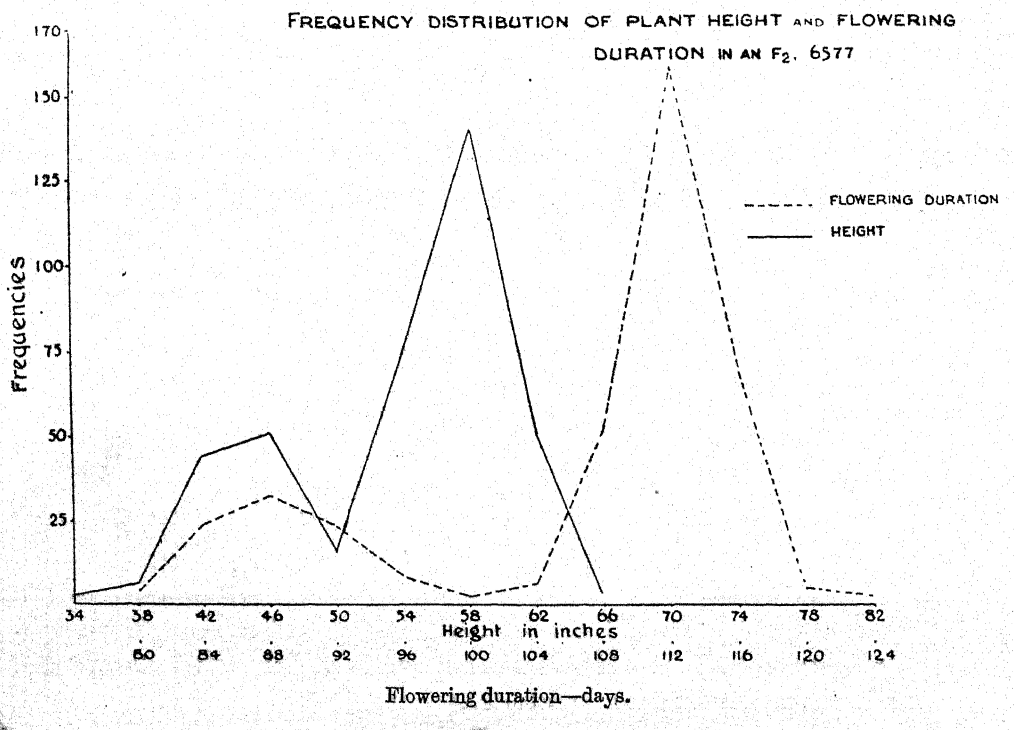

Fig. 2.

In cross 6 since both the parents are genetically the same and their behaviour, either as dominant or as recessive, is only relative to the short earlies according to whether they contain the factor E_1 or not, the F_1s and F_2s should all be tall lates. This is what was actually obtained in 2 F_2 families.

III. Discussion of results.

Thus both the factorial interpretations offered fit in with the observed F_2 results of all the six crosses. The three-factor hypothesis gives more scope for explaining the wider variability of the short earlies due to the greater number of phenotype combinations which occur in the F_2s.

The standard deviation and the coefficients of variability in the two groups of plants in all the F_2s are given in Table V.

Table V.

Mean heights and mean flowering durations of the F_2s with the parents.

No.	Cross	No.	Short early group						Tall late group					
			Heights			Durations			Heights			Durations		
			Mean height (inches)	S. D.	C. V.	Mean duration (days)	S. D.	C. V.	Mean height (inches)	S. D.	C. V.	Mean duration (days)	S. D.	C. V.
1	S. E. D. × S. E. R.	6552	39·5±·02	0·46	13·8	86·6±·39	8·64	10·0	53·6±·03	0·33	7·5	108·4±·33	3·6	3·3
		6553	38·6±·02	0·5	14·6	87·0±·38	8·8	10·1	52·7±·03	$0·3_5$	$8·_0$	108·5±·43	4·1	3·8
2	S. E. D. × T. L. D.	6558	41·0±·01	0·4	12·1	96·8±·1	5·0	5·1	52·7±·01	0·32	7·3	112·3±·15	3·9	3·4
		6559												
		6560												
		6661												
3	S. E. D. × T. L. R.	6569	41·0±·01	0·3	7·5	93·4±·2	4·5	4·8	60·5±·01	0·3	5·9	106·0±·2	3·3	3·1
		6570												
4	S. E. R. × T. L. D.	6574	41·2±·02	0·3	8·7	..	..	..	55·2±·01	0·3	6·1	..	..	..
		6575												
		6574	..	..	..	88·4±·5	5·7	6·5	..	..	..	113·8±·2	3·1	2·7
		6575	..	..	..	88·6±·5	4·9	5·5	..	..	..	112·8±·2	2·9	2·6
5	S. E. R. × T. L. R.	6577	42·4±·01	0·2	5·7	86·0±·3	3·9	4·6	55·9±·01	0·3	6·3	110·6±·1	3·3	3·0
		6578												
6	T. L. D. × T. L. R.	7472	..	..	..	..	..	..	58·3±·01	0·3	6·6	119·5±·1	$3·_9$	3·2
		7473												
	* S. E. D. parent	..	37·9±·04	..	..	90·6±·6	..	..	..	..	..	..	..	..
	Ditto	..	39·1±·04	..	..	94·4±·8	..	..	..	..	..	..	..	..
	S. E. R. parent	..	37·6±·03	..	..	87·6±·9	..	..	..	..	..	..	..	..
	T. L. D. parent	..	..	..	..	..	..	..	..	..	..	..	..	..
	Ditto	..	..	..	..	..	..	..	56·0±·04	..	..	111·5±·3	..	..
	*T. L. R. parent	..	..	..	..	..	..	..	58·7±·04	..	..	114·3±·4	..	..
	Ditto	..	..	..	..	..	..	..	59·8±·04	..	..	118·2±·5	..	..

* Grown in different fields in the same season.

It is seen that when the two groups are compared, the C. V. is always definitely higher in the short early group than in the tall late, particularly with regard to the flowering duration. The C. V. both for height and duration is the highest in cross 1 and this gives additional confirmatory evidence for the larger number of factors involved in this case.

In all the crosses described, the flowering duration and plant height were assumed to be controlled by the same factors. The high positive correlation found to exist between the two characters is the basis for such an assumption, and even if the factors are different they may remain linked which could also account for the close association of the two characters. The correlation coefficients were determined for some of the F_2s of these 6 crosses and they are all very definitely highly positive.

Cross	F_2 family numbers	Correlation coefficient between height and duration
S. E. D. × S. E. R.	6552	+ 0·74 ± ·012
	6553	+ 0·74 ± ·012
	6597	+ 0·72 ± ·015
S. E. D. × T. L. D.	6560	+ 0·74 ± ·012
S. E. D. × T. L. R.	6570	+ 0·62 ± ·034
S. E. R. × T. L. D.	6574	+ 0·73 ± ·013
S. E. R. × T. L. R.	6577	+ 0·75 ± ·015

The correlation between the two characters is also manifest in Figs. 1 and 2 giving the F_2 frequencies of the two characters. It is seen from the estimate of correlation coefficients that it is almost of the same amount in all the crosses. According to the factorial interpretation developed earlier, the segregation in the case of crosses 4 and 5 is for the presence or absence of the factor L_1 responsible for tall late, and hence the two groups of the F_2s were found to be clear-cut with hardly any overlap between the two. In the first three crosses besides the factor L_1, E_1 and E_2 also come in, making short earlies and the tall lates not all of the same factorial composition and there was consequently a slight overlapping of the two groups. The overlapping of the two groups or their clear-cut separation in the F_2s is also brought about by the peculiar distribution of the plants in the different parents. It was found from the frequency distribution of the characters that there was a definite negative correlation between height and duration in the S. E. D., T. L. D., and T. L. R. parents and a slight positive correlation in S. E. R. parent. Where the correlation between the two characters in the two groups appearing in the F_2s is of the same type, there is overlapping of the two groups, and where it is

of opposite type, as in crosses 4 and 5, there is a clear-cut separation between the groups.

IV. Behaviour of F_3.

A certain number of selections were carried forward in each of the two groups of the several F_2s. Below are given the behaviour of the F_3 selections from each cross.

Cross 1.—The short early selections can behave in any one of the following three ways :—(*i*) breed pure, (*ii*) throw tall lates in the ratio of 13 : 3 just like the F_2, and (*iii*) throw tall lates in the ratio of 3 : 1. Of these three, some families breeding pure for short early were obtained. But the number of splitting families studied were unfortunately few and the number of individuals in each family was also so small, that the two kinds of splittings, 3 : 1 and 13 : 3, could not be well made out.

Of the tall late selections some should breed pure for tall late, while others should split and give tall late and short early in the proportion of 3 : 1. There were only a few tall late selections and all of them split and threw short earlies as shown below :—

F_2s	Tall late	Short early
Totals of 4 families	370	127
Calculated 3 : 1	*373*	*124*

Crosses 2 and 3.—The behaviour of the selections in both these should be similar. The short early selections should either breed pure or throw tall lates in the ratio of 3 : 1, and the tall late selections should all breed true. Of 19 short early selections, 6 were pure short early, while the other 13 together gave

——	Short early	Tall late
	1227	430
Calculated 3 : 1	*1243*	*414*

The 19 tall late selections all bred true.

Crosses 4 and 5.—According to the interpretation given, the short early selections should all breed pure, while the tall late selections would either breed pure or throw short earlies in the ratio of 3 : 1. The eleven short early selections all bred true to the character as was expected. Of the 12 tall late selections, 5 were pure tall lates and the other 7 families together gave

	Short early	Tall late
	134	401
Calculated 1 : 3	*134*	*401*

It is thus seen that all the F_3 selections from the different crosses behaved true to the expectations.

It was stated earlier in the paper that the segregation between the groups due to the peculiar nature of behaviour of the parents with reference to the two characters, height and duration, and to the different factorial composition, was very sharp in some and was overlapping in others. In the F_2 families, where there was overlapping of the two groups, some special plants had heen selected as short lates and tall earlies to determine their F_3 behaviour. The short late selections behaved as pure short early and the tall early selections behaved as pure tall late, proving that the overlapping for the two characters was not in any way brought about by different genetic constitution. These plants were evidently types occurring at the extreme end of the fluctuating limits for the two characters. In a similar way within a group there is not a strict correlation between height and duration, *i.e.*, in a short early group the plant coming to flower early is not necessarily shorter than one that flowers later. In certain F_2 families selections were made with varying flowering durations but all with the same height, and similarly certain others, all flowering on the same date but with different heights. The F_3 behaviour of all these elections was practically identical as shown below, the mean duration and heights of the families being similar to the parental means.

1. Three selections from F_2 family 6569, with height 52 inches and duration (days) :

			87	94	100
gave in F_3	Short earlies	Mean height	46 in.	46 in.	46 in.
		Mean flowering duration (days)	98	94	97
	Tall lates	Mean height	56 in.	56 in.	56 in.
		Mean duration (days)	113	110	113

2. Three selections from F_2 family 6570, with height 62 in. but with durations in days :

			104	104	112
gave in F_3	Pure talls with	Mean height	57 in.	57 in.	58 in.
		Mean duration (days)	117	112	115

3. Three selections from F_2 family 6569, with the same flowering duration, 94 days, but with heights

			56 in.	52 in.	60 in.
gave in F_3	Short earlies	Mean height (in.)	45	46	45
		Mean duration (days)	98	95	99
	Tall lates	Mean height (in.)	53	56	56
		Mean duration (days)	111	110	114

The above results definitely prove that within the two groups the magnitude of the two characters as expressed in the F_2s is only due to their fluctuations and have nothing to do with the averages of the characters in F_3, since such fluctuation is not brought about by genetic differences.

V. Cross T. 29×T. 6.

The only cross that had been left out to get additional confirmatory evidence about the assumed genetic constitution of the parents, was that between T. 29×T. 6. This was done in 1929-30 season and the F_1s and F_2s were grown in 1930-31 and 1931-32 seasons respectively, under uniform conditions. The height of plants and the time of flowering were recorded for the individual plants in the F_2.

	Height (inches)	Duration (days)
T. 6	48	77
T. 29	54	89
F_1	55	86

It would appear from above that the character of the F_1 is just like that of the T. 29 parent, the small differences being probably due to heterosis.

F_2 results.

	Height (inches)		Duration (days)	
	Range	Mean	Range	Mean
T. 6 parent	44-54	47·3	78-96	84·8
T. 29 „	42-52	49·0	80-96	87·3
F_2	28-58	43·8	70-110	86·8

It is seen that the F_2 range in both height and duration exceed the parental limits indicating the interaction of multiple factors. There were plants that were definitely shorter and earlier than the parents and also some definitely taller and later than the parents. The assumed genetic constitution of the parents were :

	On multiple-factor hypothesis	On inhibitory-factor hypothesis
T. 6	$l_1 l_1 e_1 e_1 e_2 e_2$	l i
T. 29	$L_1 L_1 E_1 E_1 e_2 e_2$	L I

The phenotypes of the F_2 would be

On multiple-factor hypothesis			On inhibitory-factor hypothesis		
Phenotypes	Proportion	Character	Phenotypes	Proportion	Character
$L_1 E_1 e_2$	9	S. E.	LI	9	S. E.
$L_1 e_1 e_2$	3	T. L.	Li	3	T. L.
$l_1 E_1 e_2$	3	S. E.	lI	3	S. E.
$l_1 e_1 e_2$	1	S. E.	li	1	S. E.

Total short early : tall late

13 : 3 13 : 3.

The actual ratios of short early to plants taller and later than the parents in two F_2s were :

	Short early	Tall late
	1,727	**369**
Calculated 13 : 3	*1,703*	*393*

which is a good enough fit. Thus this cross has also confirmed the assumption and interpretations discussed earlier in the paper.

VI. Summary.

The study of the 6 crosses up to the F_2 and F_3 generations confirms the existence of a strong association of the two characters, plant height and flowering duration. The entire dominance of the characters of either of the parents in the F_1s indicate the simplicity of the segregation as opposed to blending of the characters usually associated with multiple factors. The genetic constitutions of the four parents, from the results of the crosses from whose progeny they were extracted, agree with the F_2 and F_3 behaviours. The clear-cut segregation or the overlap of the two groups, short early and tall late, in the F_2s have been explained as due to the genetic factorial differences of the parents and to their peculiar behaviour as regards the association of the two characters, duration and height. The obtaining of a certain number of plants in the F_2s as short late and tall early, particularly in families where the two groups overlap each other. has been shown from their F_3 behaviour to be simply due to the fluctuations of the characters and not due to any real genetic difference. The magnitude of the characters as expressed in the F_2 plants have been shown to have no relation with their F_3 behaviour proving that the inheritance was simple. Though the F_2 and F_3 results have been explained satisfactorily both on the multiple-factor hypothesis as well as on the inhibitory-factor hypothesis, the former gives wider scope to account for the wider variability arising from a greater number of pheno-type combinations which occur in the F_2s.

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REFERENCE.

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A METHOD FOR THE DETERMINATION OF CARBOHYDRATES IN LEAVES

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During the investigation of various problems connected with the physiology of photosynthesis a need for an accurate method of determining minute quantities of the different carbohydrates present in the leaves was felt by the senior author in spite of the various methods developed by different workers. The majority of these methods for the quantitative estimation of reducing sugars are based on the power of these sugars to reduce an alkaline copper sulphate solution. It was Fehling [1849, 1858] who first proposed the use of alkaline copper tartarate solution for this purpose, and since then many independent methods have been worked out by different workers like Meissl [1879], Allihn [1880], Herzfeld [1885], Hiller [1889], Soxhlet [1880, 1885], Kjeldahl [1895] and Defren [1896] for estimating d-glucose, d-fructose, invert sugar and sucrose from a mixture of sugars. Brown and Morris [1893] first employed the copper sulphate method for estimating carbohydrates in plants and they were followed by Parkin [1912] and various others. Munson and Walker [1906] unified these different methods and developed a common method for determining the reducing sugars. The gravimetric method of Munson and Walker [1906] is used by agriculturists and is recommended in the official and the tentative methods of analysis of the Association of Official Agricultural Chemists [1930]. Various sources of errors in using Fehling's solution are pointed out by Quisumbing and Thomas [1921], and methods have been devised by them to eliminate these sources of error. Schaffer and Hartman [1921] have modified the method of Munson and Walker [1906] and have developed an iodometric method for determining reducing sugars and this method is used by various workers in determining the different carbohydrates in plants. The above-mentioned methods of sugar analysis have not been found useful for determining minute quantities of carbohydrates present in a few grams of fresh leaves that are available for analysis in the work on photosynthesis. Davis and his collegues [1913-1916] have

made many improvements in the methods of extraction, purification and separation of various carbohydrates from the leaves employed by Brown and Morris [1893] and others, and they have made use of the polarimetric method of determining their quantities. The polarimetric method is also of no use in work on photosynthesis where the concentrations of sugars in the leaf extracts are very low.

Benedict and Osterberg [1918] devised a colorimetric method of determining sugars in urine in which the reducing action of sugar on picric acid in alkaline solution gives rise to a coloured substance probably picramic acid. Thomas [1924] and Thomas and Dutcher [1924] applied the picric-acid method by using a colorimeter for estimating sugars in plants. The limitations of this method were pointed out by Falk and Noyce [1920] which were taken into account by the above authors. This method was further modified by William and Davidson [1924]. The picric-acid method is accurate if various precautions are taken but it is not sufficiently sensitive as determinations of reducing sugars in solutions of lower concentrations than 0·01 per cent. can not be made with it.

Folin and Wu [1918] in estimating the minute quantities of sugars in blood made use of the fact that when reducing sugars are treated with an alkaline copper sulphate solution, the reduction of the cupric salt takes place and the cuprous oxide so formed gives a blue colouration with phosphomolybdic acid solution, any unreduced copper being at the same time decolourised. They applied certain corrections to obtain true values of sugars. Later on Calvert [1923] improved upon the technique to a certain extent and increased the accuracy of the method as well as shortened the time of estimation. Standard and Wheatley [1924] pointed out certain defects in the method of Calvert [1923], who therefore revised his method again. But on studying this revised method of Calvert [1924], it was found that it had certain defects which must be removed in order to carry out very accurate estimation of different sugars in the leaf extract. As a result of preliminary work it appeared to the writers that by making improvements in this method it was possible to make it sufficiently accurate and delicate for determining minute quantities of sugars obtained from samples of leaf-material and therefore it was undertaken to devolop this method of Folin and Wu [1918], so that it could be used for estimating the carbohydrates in the leaves. There is one serious objection to the copper sulphate method which introduces errors in the sugar estimations. It is absolutely necessary to secure uniform conditions when the reductions occur in all sugar estimations as Nef [1907, 1910, 1913] has shown that a number of products, variable in nature and quantity according to the concentrations of sugar solutions and the alkali, are formed when the reduction of alkaline Fehling's solution occurs. But this defect is greatly remedied in the procedure adopted here. As some Biochemists and Plant Physiologists have

requested the senior author to publish this method which has been developed in the laboratory during the last five years, it is undertaken to publish it separately so as to make it available to all workers in India. Though this method was originally worked by the writers, several important improvements have been subsequently made by Messrs. Chinoy, Pirzada and Asana and the improvements made by them will be referred to in the text. This method of estimating the carbohydrates in plants is used for the leaves of *Abutilon asiaticum* G. Don, *Ricinus communis* L., *Helianthus annuus* L., *Allium cepa* L., Raphanus, and Garden Stock, and stems, roots, and leaves of the rice plant (*Oryza sativa* L.)

The method of extracting the carbohydrates was the same as employed by Davis, Daish and Sawyer [1916] but some modifications had to be made according to the nature of the material extracted. It was found that the period of extracting the material varied with different leaves; but in all cases it was noticed that the period of 24 hours as recommended by Davis, Daish and Sawyer [1916] was too short for complete removal of all soluble carbohydrates from the leaves of different plants. In order to remove sugars from the rice plant it was found necessary to continue extraction with alcohol for nearly 80 to 100 hours, otherwise much of the sugar remains unextracted.

Minimum quantity of leaf material required is about 20 gms. by fresh weight and the following procedure is for that quantity.

Method of extraction.

The lamina of the leaves are separated from the stalks, weighed, cut to pieces rapidly, and thrown into boiling alcohol in a round bottomed flask containing a little ammonia. The extraction with alcohol should be continued varying from 40 to 100 hours according to the nature of the material taken for extraction. Four to five changes of alcohol during the course of extraction are necessary. The concentration of alcohol used for killing the leaves is 95 per cent. while in each successive change the concentration is lowered to 85 per cent., 80 per cent. and 75 per cent. It is known that sugars are very slightly soluble in absolute alcohol and so after the removal of chlorophyll, weaker and weaker alcohol is used to facilitate the extraction.

All the four extracts are mixed together and stored in a glass-stoppered bottle and put aside for further treatment. This alcoholic extract from the leaves cantains all soluble materials such as sugars, glucosides, tannins, albuminoids, amino acids, chlorophyll and other leaf pigments. The extract is then transferred to a distilling flask (1,000 c.c. capacity) and alcohol is allowed to distill off at 80°-81°C. and the residual matter of the leaves is evaporated to dryness in a porcelain dish on a water bath and weighed. The residue is then removed to a 1,000-c.c. beaker

with distilled water. After making the volume to 750 c.c. a solution of basic lead acetate is added to it drop by drop, to precipitate the glucosides, tannins, albuminoids, amino acids, chlorophyll and other leaf pigments. It is recommended to use the basic lead acetate manufactured by the same company in all the extractions of sugar. Gill, Pellec and Edson [1926] have pointed out that basic lead acetate precipitates levulose, but, if the solution of sugars is sufficiently dilute, if the excess of basic lead acetate is avoided, if it is not kept in contact with the sugar solution for a long time and if it is not warmed, no levulose is lost. The use of neutral lead acetate is recommended by them in place of basic lead acetate, but it is possible that neutral lead acetate may leave optically active gummy substances in the solution.

There has been a great deal of difference of opinion about the use of deleading agent to remove the excess of lead acetate after the impurities are precipitated with basic lead acetate and after the solution is filtered. The work of Bryan [1908] and Geerlings [1908] has shown that loss of reducing sugars, especially fructose, occurs when basic lead acetate is used. Davis [1916], on the contrary, contends that in dilute solution basic lead acetate, in presence of other salts, does not precipitate fructose. Doerr [1916] and Pellet [1916] have shown that reducing sugars are entrained between the particles of insoluble lead salts, as, on decomposing the lead precipitate, the entrained sugars are recovered. Similar results are obtained by Meade and Harris [1916] by using neutral lead acetate. English and Tsang [1922] made a comparative study of different deleading agents like potassium dichromate, oxalate, sodium carbonate, disodium phosphate, tannin, etc., to determine the best deleading agent for the removal of excess of lead acetate, so as to avoid loss of sugars, and they have found disodium phosphate as the best deleading agent necessary to wash the lead precipitate with water several times to remove the entrained sugar particles. The loss of sugar is below one per cent. if the necessary precautions are taken. The filtrate is again refiltered and concentrated in a dish on a water bath to a small volume and filtered for the last time, the volume is made up to 100 c.c. and is stored in a clean and sterilized glass-stoppered bottle with 4 or 5 c.c. of toluene to prevent the growth of fungus.

The leaf material left out after the extraction with alcohol is dried in the oven at 80°-85° C. for about 24 hours, cooled, powdered and weighed. It now contains starch, gummy substances and other products that are insoluble in alcohol. Davis and Daish [1914] used these leaves directly for the estimation of starch. The gummy substances, tannins and proteins and other products cantained in these leaves may pass, to a certain extent, into the solution when the leaves are boiled with water for gelatinizing starch, and may introduce an error in the end in the reducing power of the solution. Basic lead acetate would clarify the solution, but

sufficient impurity would remain to introduce an error in the analysis in some cases. This could, however, be remedied by the following method adopted by Brown and Millar [1926]. 200 c.c. of distilled water are added to the leaf powder and the beaker is incubated for 24 hours at 37°-38° C. Water is decanted off and the leaf material is washed with fresh distilled water. This helps to remove the gummy substances which, if present, would vitiate the result.

This diastase method of O'Sullivan [1884] for the hydrolysis of starch works pretty accurately when applied to the solutions of purified starch, but it does not work with satisfaction when starch of the leaf material is to be hydrolysed. The reason is that dextrin is carried down together with the precipitate of gummy substances and other products when basic lead acetate is added to it to purify the solution, and is thus lost to the analysis.

Taka diastase has been found to be more suitable than the ordinary diastase in the hydrolysis of starch from the leaf material and is used by Stone and Wright [1898], Croft Hill [1901], Davis and Daish [1914], Horton [1921] and others. It has been found by them to give rise only to maltose and dextrose free from dextrin. This fact makes the application of takadiastase method to the leaf material most suitable as there will be no loss of sugars when basic lead acetate or other clarifying agent is added to it.

Most probably the first action of taka diastase is to break down the starch to dextrin and maltose as in the case of ordinary diastase. Dextrose is formed comparatively slowly so that after six hours only one-tenth of the original starch is present as dextrose. Kita's [1913] view, that dextrose is the direct product of the hydrolysis of the starch and is not formed from the intermediate maltose, is highly improbable. Perhaps the reaction proceeds thus : Starch → dextrin → maltose → dextrose. As more and more time elapses the amount of dextrose in the solution increases and that of the maltose decreases. But after 48 hours an actual loss in values of dextrose and maltose occurs as is suggested by Davis and Daish [1914]. The optimum temperature for taka diastase is 55° C., but at such a high temperature maltose is slowly destroyed. The values of starch can be obtained by multiplying the value of dextrose by 0·9.

For the hydrolysis of starch in the leaf powder hydrochloric acid cannot be used as a number of workers like Noyes *et al.* [1904] have shown that the loss of glucose amounting to 1·0-1·5 per cent. occurs as a result of use of 2·5 per cent. of hydrochloric acid. These findings of Noyes [1904] have not been confirmed by Olmsted [1920] and Walton and Coe [1923]. O'Sullivan [1884] developed a method of estimating starch in cereals by hydrolising it by means of diastase.

The leaf powder is then boiled with 500 c. c. of distilled water on a water bath for 10 to 20 hours to gelatinise the starch. It is cooled to 35°-37° C. and 0·05

grm. of solid taka diastase (Park Davis and Co.) is added to it. The beaker is kept in the incubator (Hearsen and Co., Ltd., London) at 38° C. for 48 hours or more. Care is taken to stir the solution and to add a few c. c. of toluene every 4 or 5 hours to prevent fungus growth. At 38° C. starch could very easily be hydrolysed completely during a period of 40 to 44 hours. To ascertain whether all the starch present in the leaves was hydrolysed or not, iodine test is applied macroscopically as well as microscopically from time to time. After the completion of hydrolysis the liquid is decanted off into another beaker and the leaves are squeezed in a presser specially made for the purpose. The leaves are moistened with distilled water and pressed several times, thus making sure that no liquid remained adhering to them. The liquid together with the washings is then boiled on a water bath for half an hour to kill taka diastase present in it. It is filtered, cooled and clarified in the usual manner by adding basic lead acetate and precipitating the excess of lead by disodium phosphate. The solution is concentrated on a water bath, filtered, and the volume of the solution made up to 100 c.c. and sampled, as usual, in a glass-stoppered bottle with 4 or 5 c. c. of toluene.

One difficulty which is to be encountered in the use of taka diastase in the present investigations is that taka diastase which is used as a catalyst has the power of reducing alkaline copper sulphate solution. This fact would introduce an error in the true values of reducing sugars obtained as a result of starch hydrolysis. In order to avoid this error a correction for the reducing power of the same quantity of taka diastase used in starch hydrolyses should be applied after determining its reducing power by previous blank experiments. It was found that 1 grm. of taka diastase has got the reducing power equal to that of 0·3803 grm. of dextrose.

It was later found by Mr. Pirzada working in the laboratory that the correction made for the reducing properties of taka diastase introduced an error in the estimation of starch instead of removing it. It was also found out by the same worker that the reducing power of taka diastase varied according to the quantity of basic lead acetate solution added to the solution after the hydrolysis of starch to precipitate the proteins and fatty substances. The following method is adopted to determine the correction to be applied for the reducing power of taka diastase when different quantities of basic lead acetate are used.

0·10 grm. of taka diastase is dissolved in 500 c.c. of distilled water and heated on the water bath for half an hour to kill the enzyme as is done in the case of the hydrolysis of starch. Five such solutions of 0·1 grm. of taka diastase in water are prepared and to each one of them different quantities of basic lead acetate varying from 10 to 30 c.c. of 10 per cent. solution are added and the lead precipitated either by sodium carbonate or disodium phosphate as the case may be. The solutions are then filtered and precipitate is washed with water and the

reducing power of the filtrate determined. It was found that the reducing power of taka diastase decreased from 0·00685 grm. of glucose to 0·00344 grm. of glucose as the quantity of lead acetate added increased. It is therefore necessary to determine the reducing power of taka diastase according to the quantities of basic lead acetate used and then make the necessary correction in the value of starch in terms of glucose obtained.

Estimation of sugars.

Two standard solutions, A and B, are prepared according to the method of Calvert [1924].

(*A*) *Alkaline* $CuSO_4$ "*solution*".

Seventy grms. of pure anhydrous sodium carbonate (Merck's product) are dissolved in about 200 c.c. of distilled water in a litre flask. 13·125 grms. of tartaric acid are added to it, and the solution is stirred till the effervescence ceases, 7·824 grms. of pure crystalline copper sulphate are then dissolved in it without heating. The solution is made up to one litre by adding distilled water. This solution is kept in dark in a coloured bottle. If any sediment occurs during the first week, the clear solution is transferred to another bottle and kept corked.

(*B*) *Phosphomolybdic acid solution.*

Thirty-five grams of pure molybdic acid (Merck extra pure) are dissolved in 200 c.c. of 10 per cent. solution of sodium hydroxide and 200 c.c. of water are added to it. The whole solution is now boiled for an hour until all the traces of ammonia are driven off. It is cooled and diluted to about 350 c.c. with distilled water and 125 c.c. of 85 per cent. phosphomolybdic acid are added and the volume is made up to 500 c.c. with distilled water.

Two c.c. of the solution (B) would render 2 c.c. of the solution (A) colourless.

Two c.c. of the sugar solution are pipetted into a test tube and 2 c.c. of copper sulphate solution are added to it. The test tube, corked with a rubber cork having a bore for the vapour to pass off and to minimise the effect of oxidation on the reduced copper, is kept into a vigorously boiling water bath exactly for six minutes. 2 c.c. of phosphomolybdic acid are added to it as quickly as possible when it is taken out from the water bath. It is allowed to stand for four minutes and then cooled under the tap exactly for one minute. Intense blue colour is produced, the depth of which is proportional to the concentration of the reducing sugars present in the sugar solution under examination. The blue colour is then matched with the blue colour of a standard solution, between half an hour and one hour after the colouration is produced.

Standard sugar solution.

For the preparation of a standard sugar solution one grm. of anhydrous glucose (M. P. 146° C.) was dissolved in 100 c.c. of distilled water and kept in a glass-stoppered bottle with a few c. c. of toluene added to it. From this, standard sugar solutions of required strengths were prepared from time to time by dilution with distilled water.

The depth of the colour obtained from the standard solution should be within the range of matching in the colorimeter, *i.e.*, the ratio between the known and the unknown solutions should be between 0·8 and 1·25 on the scale of colorimeter. In order to bring the standard solution within the range of matching with the unknown solution it is essential that a fresh sugar solution is taken, diluted and treated with the copper sulphate solution. If the standard solution after the development of colour is found more concentrated than the unknown solution, former should not be diluted with water as depth of colour is not proportional to dilution after the colour is once developed. So a fresh standard solution properly diluted as required should be taken.

Matching the unknown solution with a suitable standard is a somewhat difficult task, but after a little practical experience is gained in the line, it can be done after one or two trials.

The colorimeter used in these investigations is the Nepholometer Colorimeter manufactured by " Klett Manufacturing Co., Inc. New York." Before the instrument is used as a colorimeter the following points should be noted :—(*a*) Zero point of the scale should be properly adjusted, (*b*) The adjustment of the mirrors reflecting the light into the colorimeter cups should be carried out, so that both the fields are equally bright.

Before use, the cups and the plungers are thoroughly washed three or four times with distilled water. Finally they are rinsed with a little of the solution that is to be used in the respective cups before filling them. In all the readings throughout the investigations one of the cups which was marked with a sign ' L ' is used for the standard solution and is always placed on the left-hand side, while the other cup is used for the unknown sugar solution on the right-hand side.

The position of the left-hand cup ' L ' containing the known standard solution is adjusted, the scale reading being exactly ' 30·0 ' ; the position of the right-hand cup is then shifted until both the sides are equally illuminated. It is always the right-hand cup which is moved to and fro for the final adjustment of the equal illumination of the two fields. The left-hand cup is kept always fixed. Several readings with the same pair of solutions are taken and the mean of them is taken or calculation.

To find the concentration of the unknown solution.

If the reading of the known solution, *i.e.*, the standard, and the unknown solution or the sugar whose concentration is to be found out, are taken as '*a*' and '*b*' respectively, the ratio of the concentration of the standard to the concentration of the unknown will then be represented by :—

$$\frac{b}{a} \text{ or } \frac{\text{concentration of standard}}{\text{concentration of unknown}} = \frac{\text{Colorimetric reading of the unknown}}{\text{Colorimetric reading of the standard}}$$

Thus if the concentration of the standard is known, that of the unknown can easily be found out from the colorimetric readings.

Accurate determination of sugars makes it quite imperative that all the requisite conditions, attending the heating of the standard and unknown sugar solutions, are fulfilled. The test tubes used for boiling the standard and the unknown sugar solution should have the same volume and should be of the glass of the same specific heat, because the reduction of the copper salt depends upon the internal temperature of the tubes. If the two tubes are not made of the glass of the same specific heat, the time taken for the reduction of copper salt would be different for both, when kept together in the boiling water for a fixed period of time. To find out such pairs of test tubes having the same specific heat a number of them are taken and are tried in pairs by producing the blue colouration by heating sugar solutions in them with 2 c.c. of copper sulphate and then adding 2 c.c. of phosphomolybdic acid. If the colorimetric readings on both the sides of the scale agree within one or two divisions on the vernier scale, they are taken as having identical specific heats.

It is shown by Chinoy [1932] that it is necessary for accurate estimations of sugars in the unknown solutions that the concentration of sugars should not be more than one part of hexoses in 2,000 parts of water, for, if the concentration is higher there will not be sufficient copper salt for the reduction to occur and consequently the amount of reduction will be less than the quantity of sugars present. If after six minutes' heating there is absence of a greenish tint in the boiling tube containing unknown sugar solution and alkaline copper-sulphate solution, it indicates that all the available cupric ions are reduced. This could be easily noticed and the error avoided by diluting the unknown solution to one part of sugar in about 2,000 parts of water. It is also necessary to use 4 c.c. of the alkaline copper sulphate solution instead of 2 c.c. of solution as mentioned above when the concentration of sugars in the unknown solution is one part of hexoses in 2,000 parts of water and to add 5 c.c. of phosphomolybdic acid after boiling for

six minutes. It is also necessary to prepare fresh stock solutions of alkaline copper sulphate, phosphomolybdic acid and standard glucose solution every fortnight. The sugar solutions extracted from the leaves generally contain a mixture of hexoses, and sucrose. It is always noticed that maltose is never present in the leaf extracts if the material is killed in boiling alcohol. If however maltose is present in any plant extract, it involves a correction to be made in the value of hexoses determined as mentioned above, as maltose is also a partially reducing sugar and therefore the value of hexoses is lower than what is obtained in the above determination. The correction to be applied is given below after the method for the estimation of cane sugar is described.

Estimation of cane sugar.

In the sugar solution from the leaves, cane sugar needs be hydrolised and converted into hexoses. Davis and his colleagues [1916] recommend 10 per cent. citric acid for hydrolysis of cane sugar and it is necessary to boil the sugar solution for 10 minutes. Citric acid is then neutralised by sodium carbonate. In the beginning the hydrolysis of cane sugar was carried out by citric acid, but it was soon found that this method was unsuitable on account of the following reasons.

The presence of sodium citrate produced as a result of addition of sodium carbonate in the unknown sugar solution interferes with the development of the blue colour when the above method is applied, so much so that no blue colour develops when the concentration of the total reducing sugars is one part in 6,000 parts of water. Hence it is not possible to estimate cane sugar when it is present in lower concentrations than 0·02 per cent. It is possible to avoid the difficulty by concentrating the sugar solutions by boiling; but that was not suitable and the following modification was made.

Hydrolysis of cane sugar.

Tartaric acid is employed as a hydrolysing agent. It is found by Asana that 10 per cent. tartaric acid is sufficient to hydrolyse cane sugar present in the 2 c.c. of unknown sugar solution by boiling it at 100°C. for 15 minutes. The sodium tartarate that is formed on neutralizing the tartaric acid with sodium carbonate does not inhibit the development of blue colour with phosphomolybdic acid and the cane sugar in lower concentration than 5 parts in 100,000 parts of water can be readily estimated.

In all estimations of cane sugar after hydrolysis with the tartaric acid, 4 c.c. of the alkaline copper sulphate solution and 10 c.c. of phosphomolybdic acid are used. It may be mentioned that the same quantities of the two solutions A and B, when citric acid is used as a hydrolytic agent, do not produce the blue colour with the lower concentrations of cane sugar than 0·02 per cent.

The difference between the concentrations of reducing sugars before and after the hydrolysis with 10 per cent. tartaric acid will give the concentration of cane sugar as glucose present in the sugar solutions prepared from the leaves. In order to obtain the true value of cane sugar it is necessary to multiply the glucose value of cane sugar by 0·95 as one gram of glucose corresponds to 0·95 gram of cane sugar.

If '*a*' represents the concentration of reducing sugars in the sugar solution from the leaves, and '*b*' represents the concentration of reducing sugars after hydrolysis with the tartaric acid,

a=hexoses in the leaves

$(b-a)\times 0{\cdot}95$ = cane sugar in the leaves.

The sugar solutions from the leaf extract is slightly coloured yellow in some cases and hence the blue colour obtained with phosphomolybdic acid becomes faintly green. Calvert [1924] also obtained greenish colour from the unknown solution of the blood sugar and in order to meet the difficulty due to the absence of any yellow colour in the standard glucose solution he recommended the use of a series of glasses of blue and yellow colour to match with the greenish colour obtained with the unknown sugar solution. The use of coloured glasses for matching the colours of the two solutions is not satisfactory and therefore a few drops of phenol red are added to the standard sugar solution and then the standard and the unknown sugar solutions are matched in the colorimeter till they are of the same tint. Phenol red has several advantages over the other colouring matters. It gives the same tint of colour as that of the unknown solution. It is neither an oxidising nor a reducing agent and it does not give a precipitate with the alkaline copper sulphate solution or the phosphomolybdic acid solution or with the mixture of the two.

It is necessary to determine the quantity of phenol red to be added before treatment with the solutions A and B, by a blank experiment. The determined quantity of phenol red is then added to the known standard sugar solution after the blue colour is developed just before comparing the two solutions in the colorimeter.

The sugar solutions from the leaves, besides the yellow colour mentioned above, contain many other impurities some of which have an inhibitory effect on the reducing power of sugar solutions, and hence the values of sugars obtained will be lower than the true values. Folin and Wu [1918] and Calvert [1923] used sulphuric acid, sodium tungstate and other substances for obtaining sugar solutions from blood and they compared the sugar solutions containing these impurities with pure glucose solution in a colorimeter. Calvert [1924], and Standard and Wheatley

[1924], who criticised Calvert's paper, did not add the same impurities to the standard glucose solution in order to obtain the true values of sugars. Davis and Daish [1913] observed the retarding effect of sodium acetate on the hydrolysis of cane sugar with 2 per cent. citric acid, and they therefore recommended the use of 10 per cent. citric acid for the cane sugar hydrolysis.

The sugar solutions from the leaf extracts contain the following impurities: (1) Sodium acetate formed in the double decomposition of the basic lead acetate and the deleading agent like sodium carbonate or disodium phosphate. (2) Sodium carbonate or disodium phosphate added in slight excess. (3) When citric acid or tartaric acid are used for hydrolysis of starch and when they are neutralised by sodium carbonate, sodium salts of these acids are present in the sugar solutions. (4) If maltose is present in the sugar solutions it is hydrolysed with sulphuric acid and so sodium sulphate remains in the sugar solutions as an impurity.

The presence of these salts in the sugar solutions interferes with the development of blue colour in the sugar solutions. The inhibitory effect on the depth of blue colour is greater in the presence of sodium acetate, sodium citrate and sodium tartarate than when sodium sulphate and sodium chloride are present. This can be explained by the fact that the former are salts of a strong base with weak acids and the latter are salts of strong bases with strong acids. The former, when they dissociate, liberate acetate and citrate ions which may be responsible for the inhibition of blue colour.

The concentrations of these impurities must be determined in order to add the same in the same amounts to the standard glucose solution.

The concentration of sodium acetate and sodium carbonate in the sugar solution obtained from the leaves can be determined by titrating with phosphomolybdic acid using thymol blue as an indicator. Two to three drops of thymol blue added to the 2 c.c. of sugar solution will produce blue colour due to the presence of sodium carbonate. Drops of phosphoric acid are added to the sugar solution till the blue colour disappears. The amount of phosphoric acid is equivalent to the sodium carbonate present which can be determined by titrating a known volume of sodium carbonate against the same amount of phosphoric acid solution using the same indicator in the same amounts.

More phosphoric acid is then added to the same 2 c.c. of sugar solution till it turns permanently pink. The amount of additional phosphoric acid will be equivalent to the amount of sodium acetate present in the sugar solution. The quantity of sodium acetate can be determined by titrating a known volume of it against the same amount of phosphoric acid using the same indicator in the same amount.

The quantities of sodium acetate and sodium carbonate thus determined should be added to the standard sugar solution. These determinations should be made in each experiment.

In the case of sugar solutions treated with tartaric acid for hydrolysis of cane sugar the same quantity of tartaric acid should be added to the standard sugar solution and neutralised by sodium carbonate before comparison in the colorimeter.

Estimation of maltose.

When maltose is present in the sugar solutions from plant extracts the value for reducing sugars' *a* ' obtained before hydrolysis with tartaric acid does not give true values of hexoses present as a part of the value is due to reducing action of maltose. It is known that one gram of maltose has the reducing power equivalent to 0·62 gram of glucose. When one gram of maltose is hydrolysed with sulphuric acid it gives 1·05 grams of glucose. It therefore follows that the reducing power of maltose after hydrolysis with acid is increased by 1·05—0·62=0·43 gram of glucose. Therefore if reducing power of the sugar solution after hydrolysis is equal to 0·43 gram of glucose, one gram of maltose is present in the sugar solution. It therefore follows that one gram of glucose is equivalent to 2·32 grams of maltose.

The glucose value of maltose should therefore be multiplied by 2·32 to get the true value of maltose. To 2 c. c. of the sugar solution from the leaves is added concentrated sulphuric acid so as to make a 10 per cent. solution of sulphuric acid. It is heated in a water bath for 10 minutes at 70°C. The acid is then neutralized with sodium carbonate. The standard glucose solution is also treated in the same manner and the necessary amounts of sodium acetate and sodium carbonate are also added as described above. The estimation is then carried out in the usual manner.

Let the total reducing sugars estimated after hydrolysis with sulphuric acid be represented by ' *c* '. Then

$(b-a)\times 0{\cdot}95$=cane sugar as shown above, and

$(c-b)\times 2{\cdot}32$=maltose, and

a—(maltose$\times 0{\cdot}62$)=hexoses.

It is known that tartaric acid can hydrolyse only cane sugar while sulphuric acid hydrolyses both cane sugar and maltose. If maltose is absent in the sugar solutions from leaves, the reducing power of the sugar solution after hydrolysis with the tartaric acid should be the same as the reducing power obtained after hydrolysis with sulphuric acid. The reducing power of the sugar solution should increase after hydrolysis with sulphuric acid if maltose is present. It was observed several times that in some cases there was no increase in the reducing power by sulphuric

acid hydrolysis over the reducing power obtained by the tartaric acid, while in some sugar solutions there was a rise in the reducing power after sulphuric acid hydrolysis. This difference was first attributed to the presence of maltose. But if maltose is present, 2/3 rds of it should be accounted for in the first determination of reducing sugar before hydrolysis with tartaric acid. In no case the reducing power of the 2/3 rds maltose could be realized from the first reading as the reducing sugars determined before hydrolysis with tartaric acid were too small in amount, and it is not possible to subtract the value of 2/3 rds of maltose from " a " as $(c—b)\times 2{\cdot}32\times 0{\cdot}62$ is always much greater than ' a ' in all cases.

Therefore maltose is absent in the sugar solutions from leaves.

It was also thought that the increased reducing power of sugar solutions after hydrolysis with sulphuric acid may be due to imperfect hydrolysis of cane sugar with citric acid, but blank experiments with pure cane sugar solutions negatived this idea.

The increased reducing power of the sugar solutions on hydrolysis with sulphuric acid may possibly be due to the presence of gummy substances or glucosides which are not completely precipitated from it by basic lead acetate. Rosenthaler [1930] also remarks that sometimes even after precipitation with basic lead acetate glucosides are present. It is not clearly understood why the glucosides and gummy substances should remain unprecipitated in some experiments while not in others as the procedure adopted is the same in all cases. If maltose is absent which can be easily seen as explained above, there is no danger of an error in the estimations as the increase in the reducing power by the sulphuric acid hydrolysis should be neglected, but when maltose is present in plant extracts, the real difficulty arises as the estimated value of maltose will be higher than its true value if the gummy substances remain partly unprecipitated. This would also introduce an error in the calculations of the value of glucose.

The Bancroft's reagent is supposed to be reduced by maltose only and not by hexoses. If this is true, it would be possible to find out the correct value of maltose without hydrolysis with sulphuric acid, by deducting 2/3 rds the value of maltose obtained from the value of 2/3 rds maltose and hexoses (a) by the method described above. Bancroft's reagent was prepared but on repeated trials it was found that glucose also reduced the Bancroft's reagent and hence it was not possible to determine the value of maltose separately.

Estimation of starch.

Starch is hydrolysed to dextrose and maltose by taka diastase as described above. So the solution contains a reducing sugar, dextrose, and a partially reducing sugar, maltose. Maltose is therefore hydrolysed with 10 per cent. sulphuric acid at

70°C. and the total reducing sugars are estimated in the colorimeter. The value of starch is obtained by multiplying the value of dextrose obtained by 0·9.

In the following appendix is shown how the different sugars present in leaves are calculated from an actual experiment.

Helianthus annuus L.

Fresh weight of the leaves=20 grams.

Volume of the sugar solutions from the leaves=125 c.c.

Impurities=4 drops of sodium acetate in 2 c.c. of sugar solution ; 1 drop of sodium carbonate in 2 c.c. of sugar solution.

Yellow colour=one drop of phenol red. These are added to the standard glucose solution.

Standard=1 gram of glucose in 7000 c.c. of water.

Colorimetric readings (before hydrolysis).

	Standard sugar solution	Unknown sugar solution	Standard / Unknown	Concentration of the unknown in 100 grms. of leaves
1st reading	31·0	25·0	124·3 / 100·0	$\frac{1·243}{7000} \times \frac{125}{1} \times \frac{100}{20}$ =0·1110 gram.
2nd reading	37·0	30·0		
3rd reading	25·0	20·0		
4th reading	18·9	15·0		
5th reading	12·4	10·0		
	124·3	100·0	...	...

Hydrolysis with 10 per cent. tartaric acid for 15 minutes.

Standard solution=1 gram of glucose in 5000 c.c. of water.

	Standard	Colorimetric unknown	Standard / unknown	Concentration of the unknown in 100 grms. of leaves
1st reading	35·2	30·0	1·175	$\frac{1·175 \times 125}{5000} \times \frac{100}{20}$ =0·1469
2nd reading	23·5	20·0		
3rd reading	47·0	40·0		
4th reading	11·8	10·0		
5th reading	...	...		
	117·5	100·0	...	...

Therefore cane sugar=(0·1459—0·1110)×0·95=0·00341 gram.

Hydrolysis of the same sugarsolution from the leaves with 10 per cent. H_2SO_4:—

Standard=1 gram of dextrose in 5000 c.c. of water.
Colorimetric readings same as those after tartaric acid hydrolysis.
Therefore concentration of unknown =0·1469
Therefore maltose is absent.

Sensitivity of this method.

According to this method reducing sugars in a concentration of 0·001 per cent. *i.e.* one part of a reducing sugar in 100,000 parts of water can be accurately determined. Similarly starch after its hydrolysis to reducing sugars can be determined in that concentration.

On account of the inhibiting influence of citric or tartaric acid on the development of blue colour, cane sugar can be accurately determined in a concentration of 0·005 per cent. *i.e.* one part of cane sugar in 20,000 parts of water.

Experimental results.

The detailed results obtained by this method have already been published in this Journal in a previous contribution by Dastur and Chinoy [1932] on the carbon dioxide assimilation of the leaves of the rice plant.

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Summary.

The method of Folin and Wu [1918] and subsequently improved upon by Calvert [1923, 1924] for estimating sugars in blood is modified and developed for estimating carbohydrates in leaves. Various sources of error in the methods devised by the above-mentioned workers are removed and various improvements described below are made.

The sugar solutions from the leaf extracts contain a mixture of simple sugars and cane sugar. Maltose is generally absent in the leaves. The hexoses are deter-

mined by matching in a colorimeter the blue colour produced when 2 c.c. of the sugar solution is treated with 2 c.c. of the alkaline copper sulphate solution and 2 c.c. of the phosphomolybdic acid solution with the blue colour produced in 2 c.c. of a standard glucose solution treated in a similar manner. By this method reducing sugar in a concentration of 0·001 per cent. can be accurately estimated.

For estimating cane sugar generally 10 per cent. citric acid has been used for its conversion into reducing sugar. It was noticed here that sodium citrate inhibits the blue colour produced, and, it is therefore not possible to estimate cane sugar in a lower concentration than 0·02 per cent. when hydrolysed with citric acid. For hydrolysis of cane sugar 10 per cent. tartaric acid is used.

Corrections to be made for various impurities present in the sugar solutions from the leaves are worked out and described. Maltose is absent in the leaves. Sometimes gummy substances and glucosides remain unprecipitated by basic lead acetate which, when hydrolysed by 10 per cent. sulphuric acid, are incorrectly taken as maltose.

A method of estimating hexoses and maltose in presence of the latter is described as maltose is also a partly reducing sugar.

Starch is estimated as dextrose first by hydrolysis with taka diastase into dextrose and maltose, and then the hydrolysis of maltose by 10 per cent. sulphuric acid again into dextrose.

Various precautions to be taken such as the specific heat of boiling tubes, the maximum concentration of the unknown solution to be taken, the quantities of the alkaline copper sulphate solution and phosphomolybdic acid to be used, the time for boiling the solutions and the arrangement of the colorimeter with the range of matching the colours of the unknown and known sugar solutions are described.

The depth of blue colour produced is not proportional to dilution with water, so the dilution of the solution after the development of the blue colour for bringing it within the range of matching with the other solution must be avoided.

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THE CLASSIFICATION OF BURMESE SESAMUMS (*SESAMUM ORIENTALE* LINN.)

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(With Plate XXXI).

INTRODUCTION.

In 1930-31 there were 1,321,959 acres of sesamum (*Sesamum orientale* Linn., syn. *S. indicum* DC.). in Burma, the largest area of any province in India and the second largest area of any crop in Burma. It was made up of 990,294 acres of 'early'* and 331,665 acres of 'late' sesamum. A full account of the sesamum crop in Burma has been given by McLean [1932].

In 1923 a collection of sesamum varieties was made from all over the province and a study of the types commenced. At first only early types were dealt with but since 1929 the late types also have been under selection.

EARLY AND LATE CHARACTERS.

It is necessary to make a clear distinction between the early monsoon variety (Burmese, *hnanyin*) and late monsoon variety (Burmese, *hnangyi*). Kashi Ram [1930] does not appear to have observed that there are two distinct classes of sesamum, one which is generally grown in the early part of the monsoon from April to the end of June and one which is always grown in the cold season being planted in late September or October and harvested in December or January. In fact, the flowering dates given for his late types (end of October) approach the flowering dates of the cold weather type rather than the early monsoon type so that it is probable that his crops were grown too late to observe the distinction. McLean [1932] writes "There is an important distinction between these two types of 'hnanyin' and 'hnangyi'. The former can be grown either as early or as late sesamum.

*The words early and late in this paper are used to denote the types grown in the early monsoon (April to June) and late monsoon (September to November) and not to indicate life-period.

Late. Early.

Fig. 1.—F_2 Hybrids.

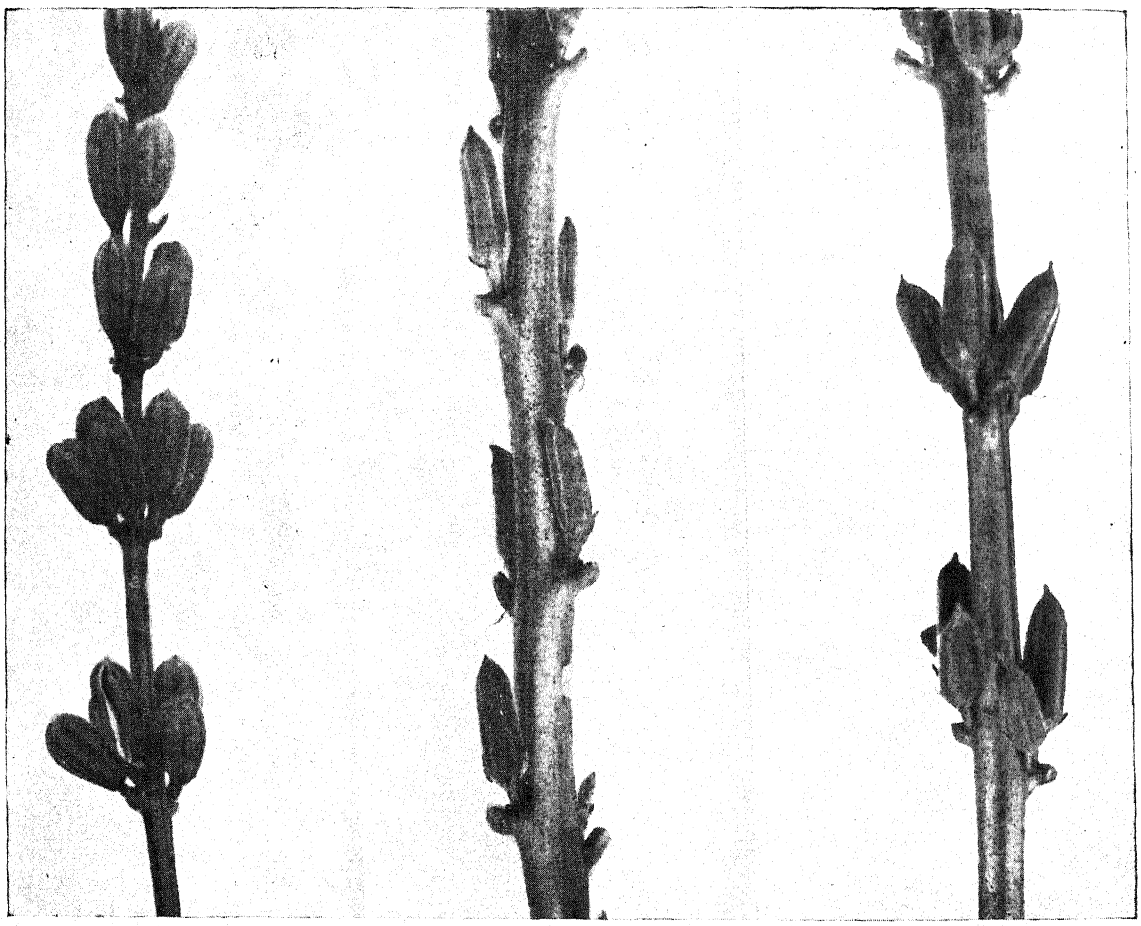

Fig. 2.—Types of capsule arrangement.

The latter is strictly a cold season crop producing, when sown early in the monsoon, much vegetative growth but failing to bear flowers and fruit." With this we are in agreement except that occasionally abortive flowers may be produced.

Inheritance of early and late characters.

In 1930 by growing both early and late types in the cold season crosses were made. In 1931 part of the F_1 was grown in the monsoon season, some at Mandalay and some at Tatkon. Both crops were identical in that the late character was dominant. The remainder of the F_1 was grown in the late season to produce seed and this was sown in the early season of 1932. At Mandalay the crop failed but at Tatkon a fair stand was obtained and the following counts made :—

Table 1.

F_2 of Thadunbyu 30—1 (late) × Pyegyi 25—135 (early).

	Late		Early	
	Number	Per cent.	Number	Per cent.
Observed	2214	74·25	761	25·75
Expected (3 : 1)	2231·25	75·00	743·75	25·00
Actual error	..	·75	..	·75
S. E. of expectation	..	·79	..	·79

There seems little doubt that one principal pair of genes is concerned though the occurrence of a few somewhat intermediate types, here classified as 'lates,' suggests that other modifying factors may also occur. In Plate XXXI, fig. 1 are represented two hybrid F_2 plants, the one on the left showing the profuse vegetative growth of a late type when grown in the monsoon. The F_1 showed some heterosis in all cases.

Differentiating characters.

There is much variation in the morphological characters of the sesamum plant, a single character often varying on the same plant or even on the same branch. This occurs in such characters as the shape of the leaves, the position of the flowers, the number of fruits per axil and the number of loculi per capsule. This classification is based on seed colour, branching habit, number of flowers per axil, number of loculi per capsule and hairiness of capsule. In spite of the variability which some of these characters exhibit it is not difficult to determine to which type a plant belongs because variation occurs mainly on the lower or upper parts only and consideration of the main form decides the classification.

(*a*) *The branching habit.*—The number of branches produced by plants of different varieties of sesamum varies widely. In some varieties most of the plants have no

branches, while in a few of the more vigorous plants one or two pairs of branches may be formed in the axils of the lowest leaves. In the branched varieties, branches are formed right up the main stem with only a small part of it left for the production of the flowers of the terminal inflorescence. These branches again produce secondary branches ending in inflorescences. Varieties intermediate in branching habit between these two extremes occur and these are included in the branched group in the classification. As the degree of branching affects the life-period of the plant, this variation in branching habit is one of the most important characters in Burmese sesamums. The unbranched varieties flower early and the life-period is short. They succeed in the dry tracts where rainfall is scanty and uncertain, conditions under which the branched types with a longer life-period would fail. The latter varieties, however, are suitable for places where the rainfall is good or where the soil is retentive of moisture.

(*b*) *Number of capsules.*—Generally one flower with two yellow extra-floral nectaries are borne in each leaf-axil. In some types, however, one or both of the these develop into flowers which set fruit, so that plants bearing one, two or three capsules occur, in the last two cases producing a whorled appearance. In certain types the lower axils bear only one capsule each, while the upper ones bear two or three (Plate XXXI, fig. 2).

(*c*) *Number of loculi.*—The number of loculi in a capsule may be four, six, eight or occasionally ten. Capsules having different numbers of loculi are met with on the same plant and also in the same axil of the whorled types, the central capsule of which may be of six or eight loculi while the lateral ones have only four each. These are classified as multilocular. Kashi Ram [1930] states that types with more than one multilocular capsule per axil never occur, but they occur in Burma, *e.g.*, Type 7 (Plate XXXI, fig. 2).

With increase in the number of loculi the length of the capsule often decreases. Nevertheless the capsules having a greater number of loculi produce most seed.

(*d*) *Seed-coat colour.*—Black, white, yellow, reddish brown and drab are the commonest colours. Other colours less frequently met with are grey, olive green, dark brown, etc. An attempt to elucidate the inheritance of testa colour was not successful. In 1928 a black early was crossed with a white. The F_1 was black and heterosis noticeable. No precautions against natural crossing in the F_1 were taken and the F_2 produced a large range of colours which defied classification.

McLean [1932] has dealt with the commercial preferences as regards seed colour.

(*e*) *Hairiness of capsules.*—All types bear longer or shorter hairs on the capsules and other parts. The character is variable but there is little difficulty in dividing

it into two groups, "hairy," having abundant long hairs and "slightly hairy," having fewer short hairs.

Sepaloid condition.

One of the commonest complaints of Burmese sesamum is that known as "*pothe*" in which the flowers are modified in various ways leading to the abortion of the ovules or the complete suppression of the floral organs or both. Descriptions of the morphology of sepaloid flowers have been given by McGibbon [1924] and Roy [1930]. The percentage of affected plants varies with the type and season and so is to some extent capable of being selected out. Odell [1925] records that seed from the lower capsules of affected plants did not show as much sepaloidy in the progeny as seed from normal plants of the same strain. The condition is most common amongst unbranched types [see under Association of Characters].

Measurements.

During the season 1924, measurements of the height, size of capsules, number of capsules per plant, etc., were made in every strain under observation. Similar measurements were also made in their progeny in the following season. The average figures of three strains in each class for the years 1924 and 1925 are given below :—

Table II.

Measurements of branched and unbranched early sesamums.

Type		Height cm.	No. of capsules per plant	Size of capsules			Life-period in days
				Length cm.	Breadth cm.	Thickness cm.	
Unbranched, 4 loculi per capsule	1924	107	81	2·7	0·8	0·6	83
	1925	117	75	2·7	0·8	0·6	85
Unbranched, 8 loculi	1924	112	56	2·9	1·1	1·1	84
	1925	99	64	2·5	1·0	1·0	85
Branched, 4 loculi	1924	112	187	2·7	0·8	0·6	96
	1925	135	279	2·9	0·6	0·5	96
Branched, 8 loculi	1924	91	163	2·2	1·1	1·1	96
	1925	97	174	2·5	1·0	1·0	95

Association of characters.

By the use of four-fold tables association between various characters has been demonstrated and shown in Table III together with the values of χ^2 and P, the former being a measure of the probability of independence so that the higher the values of χ^2 the higher the degree of association, while P, the probability of independence, will in that case be very small. The full data are given in the Appendix.

Table III.

Association between characters. (Early varieties).

	χ	P
Branching and number of capsules per axil	11·6690	0·0086
Branching and seed colour	12·640	0·0055
Branching and number of loculi	0·2815	0·9507
Seed colour and number of loculi	3·8880	0·2736
Number of capsules and number of loculi	10·319	0·0172
Seed colour and number of capsules	10·9042	0·0122
Branching and sepaloidy	16·3796	0·0009

It appears that branching is associated with both seed colour and number of capsules per leaf-axil, black or dark coloured seed and single axillary capsules being commonest amongst branched types. Branching and number of loculi per capsule are independent. There is small association between seed colour and number of loculi, types with coloured seed being somewhat more numerous in the 4-locular class, while number of capsules is definitely associated with numbers of loculi and seed colour, the types with more than one capsule per axil having usually four loculi and coloured seed being commonest with the single capsules. The unbranched types are more severely affected by the sepaloid condition than the branched types.

Classification of 'early' types.

One hundred and fourty-four cultures were grown in 1924. These were selected from a larger number grown in 1923 which contained some late types.

All cultures were grown on the Tatkon farm. The characters used for classification are (1) Branching, (2) Seed-coat colour, (3) Number of capsules per axil, (4) Number of loculi per capsule, and (5) Hairiness of capsule.

The Burmese names and register numbers of the principal selections are added.

KEY TO THE TYPES OF BURMESE 'EARLY' SESAMUMS.

A.—Plants branched—
I.—Seeds black—
(1) Number of capsules—one per axil—
α.—Loculi—four per capsule—
a. Capsules hairy . . . . . Type 1
b. Capsules slightly hairy . . . . . Type 2
β.—Loculi—more than four per capsule—
a. Capsules hairy . . . . . Type 3
b. Capsules slightly hairy . . . . . Type 4
(2) Number of capsules—more than one per axil—
α.—Loculi—four per capsule—
a. Capsules hairy . . . . . Type 5
β.—Loculi—more than four per capsule—
a. Capsules hairy . . . . . Type 6
b. Capsules slightly hairy . . . . . Type 7
II.—Seeds white—
(1) Number of capsules—one per axil—
α.—Loculi—four per capsule—
a. Capsules hairy . . . . . Type 8
b. Capsules slightly hairy . . . . . Type 9
β.—Loculi—more than four per capsule—
a. Capsules hairy . . . . . Type 10
b. Capsules slightly hairy . . . . . Type 11
(2) Number of capsules—more than one per axil—
α.—Loculi—four per capsule—
a. Capsules hairy . . . . . Type 12
b. Capsules slightly hairy . . . . . Type 13
III.—Seeds drab—
(1) Number of capsules—one per axil—
α.—Loculi—four per capsule—
a. Capsules hairy . . . . . Type 14
β.—Loculi—more than four per capsule—
a. Capsules hairy . . . . . Type 15
b. Capsules slightly hairy . . . . . Type 16

(2) Number of capsules—more than one per axil—

α.—Loculi—four per capsule—

a. Capsules hairy . . . . . Type 17

IV.—Seeds brown—

(1) Number of capsules—one per axil—

α.—Loculi—four per capsule—

a. Capsules hairy . . . . . Type 18

b. Capsules slightly hairy . . . . Type 19

β.—Loculi—more than four per capsule—

a. Capsules hairy . . . . . Type 20

b. Capsules slightly hairy . . . . Type 21

B.—Plants unbranched—

I.—Seeds black—

(1) Number of capsules—one per axil—

β.—Loculi—more than four per capsule—

b. Capsules slightly hairy . . . . Type 22

(2) Number of capsules—more than one per axil—

β.—Loculi—more than four per capsule—

a. Capsules hairy . . . . . Type 23

II.—Seeds white—

(1) Number of capsules—one per axil—

α.—Loculi—four per capsule—

a. Capsules hairy . . . . . Type 24

b. Capsules slightly hairy . . . . Type 25

β.—Loculi—more than four per capsule—

a. Capsules hairy . . . . . Type 26

b. Capsules slightly hairy . . . . Type 27

(2) Number of capsules—more than one per axil—

α.—Loculi—four per capsule—

a. Capsules hairy . . . . . Type 28

b. Capsules slightly hairy . . . . Type 29

β.—Loculi—more than four per capsule—

a. Capsules hairy . . . . . Type 30

III.—Seeds drab—

(1) Number of capsules—one per axil—

α.—Loculi—four per capsule—

a. Capsules hairy . . . . . Type 31

β.—Loculi—more than four per capsule—
b. Capsules slightly hairy . . . . . Type 32
(2) Number of capsules—more than one per axil
α. Loculi—four per capsule
a. Capsules hairy . . . . . Type 33
IV. Seeds brown.
(2) Number of capsules—more than one per axil
α. Loculi—four per capsule
a. Capsules hairy . . . . . Type 34

Description of early types.

Type 1.—Plants tall (117 cm.), much branched; leaves small, light green, petioles dull red, lower leaves ovate, serrated, middle leaves lobed trifoliolately, serrated and upper leaves linear-lanceolate, entire; flowers opposite, pink, not mottled, hairy, (length of corolla) 4·1 cm. × (diameter of tube) 1 cm., capsules solitary, hairy, short, 2·9 cm. × 0·6 cm., 4 loculi, seeds black; life-period 82 days.

Type 2.—Plants medium height (107 cm.), branched; leaves small, light green, petioles dull red, lower leaves ovate, entire, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, pink, not mottled, slightly hairy, 4·1×1 cm., capsules solitary, slightly hairy, short, 2·9 × 0·6 cm., 4 loculi, seeds black; life-period 92 days. (*Allanmyo Pyegyi*).

Type 3.—Plants short (86 cm.), branched; leaves large, greyish green, petioles red, lower leaves narrowly ovate, entire or slightly serrated, middle leaves lobed deeply, serrated, upper leaves linear-lanceolate, entire; flowers alternate, white, not mottled, very hairy, very short, 2·2 × 1 cm.; capsules solitary, hairy, short, 2·5 × 1 cm., 8 loculi, seeds black; life-period 80 days.

Type 4.—Plants short (91 cm.), branched; leaves medium size, light green, petioles red, lower leaves ovate, entire, middle leaves lobed deeply, serrated, upper leaves linear-lanceolate, undulate or entire; flowers alternate, white, not mottled, slightly hairy, 3·8 × 1 cm.; capsules solitary, slightly hairy, short, 2·4 × 1 cm., 8 loculi, seeds black; life-period 93 days.

Type 5.—Plants short (91 cm.), branched; leaves large, dark green, petioles red, lower leaves ovate, slightly serrated, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, light pink, mottled, hairy, (3·8 × 1 cm.); capsules 2 to 3 per axil, hairy, short (2·5 × 0·6 cm.), 4 loculi, seeds black; life-period 80 days.

Type 6.—Plants medium height (112 cm.), medium branched, leaves large, dark green, petioles red, lower leaves narrowly ovate, serrated, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, serrated; flowers alternate, pink, not mottled, very hairy, large (4×1 cm.); capsules 2 to 3 per axil, very hairy, long (3·2×1·1 cm.), 6 to 8 loculi, seeds black; life-period 80 days.

Type 7.—Plants short (86 cm.), branched; leaves small, dark green, petioles red, lower leaves ovate, serrated, middle leaves lobed, narrow, serrated, upper leaves linear-lanceolate, entire; flowers alternate, deep pink, deeply mottled, slightly hairy, large (4·1×1 cm.); capsules 3 per axil, slightly hairy, (3·2×0·8 cm.), 6 to 8 loculi, seeds black; life-period 83 days.

Type 8.—Plants short (90 cm.), branched; leaves greyish green, petioles red, lower leaves narrowly ovate, serrated, middle leaves ovate, lobed, much serrated, upper leaves lanceolate, serrated; flowers alternate, pink or deep pink, mottled, hairy, large (4·3×1·1 cm.); capsules solitary, hairy, (3×0·6 cm.), 4 loculi, seeds white; life-period 85 days.

Type 9.—Plants short (90 cm.), branched, leaves medium size, dark green, petioles light purple, lower leaves ovate, slightly serrated, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, deep pink, not mottled, slightly hairy, (4·1×0·8 cm.); capsules solitary, slightly hairy, short (2·8×0·6 cm.), 4 loculi, seeds white; life-period 83 days. (*Gwagale* 25—6.)

Type 10.—Plants medium height (107 cm.), branched; leaves large, dark green, petioles red, lower leaves ovate, serrated, middle leaves lobed trifoliolately, much serrated, upper leaves linear-lanceolate, entire; flowers alternate, pink or deep pink, mottled, very hairy, large (4·3×1 cm.); capsules solitary, hairy, short, thick (2·9×1·1 cm.), 6 to 8 loculi, seeds white; life-period 80 days.

Type 11.—Plants short (96 cm.), branched, leaves medium size, greyish green, petioles red, lower leaves narrowly ovate, serrated, middle leaves lobed deeply to trifoliolately, slightly serrated, upper leaves linear-lanceolate, entire; flowers opposite, white to light pink, not mottled, slightly hairy, (4·1×1 cm.); capsules solitary, slightly hairy, short (2·5×1 cm.), 6 to 8 loculi, seeds white, life-period 86 days. (*Shwepothagaung* 25—82.)

Type 12.—Plants short (90 cm.), branched, leaves large, dark green, petioles red, lower leaves narrowly ovate, slightly serrated, middle leaves lanceolate, lobed, serrated, upper leaves linear-lanceolate, entire; flowers opposite and alternate, white, not mottled or deep pink mottled, hairy (4×1 cm.); capsules 2 to 3 per axil, hairy, long (3·2×0·7 cm.), 4 loculi, seeds white; life-period 80 days.

Type 13.—Plants medium height (112 cm.), branched, leaves small, light green, petioles light purple, lower leaves ovate, serrated, middle leaves broadly ovate, lobed, serrated, upper leaves linear-lanceolate, undulate; flowers opposite, white,

not mottled, slightly hairy, (4·1×1 cm.); capsules 3 per axil, slightly hairy, (2·9×0·5 cm.); 4 loculi, seeds white ; life-period 82 days.

Type 14.—Plants short (96 cm.), much branched, leaves small, greyish-green, petioles red, lower leaves broadly ovate, serrated, middle leaves ovate, lobed, much serrated, upper leaves linear-lanceolate, entire; flowers alternate, pink to deep pink, not mottled, hairy (4×1 cm.); capsules solitary, hairy, long (3·3×0·8 cm.), 4 loculi, seeds drab ; life-period 80 days.

Type 15.—Plants short (91 cm.), much branched; leaves small, dark green, petioles red, lower leaves ovate, entire, middle leaves ovate, lobed, serrated, upper leaves linear-lanceolate, entire ; flowers alternate, pink, not mottled, hairy (3·2×1 cm.); capsules solitary, hairy, short (2·5×1 cm.), 6 to 8 loculi, seeds greenish grey ; life-period 87 days.

Type 16.—Plants tall (115 cm.), branched; leaves small, dark green, petioles red, lower leaves ovate, slightly serrated, middle leaves deeply trifoliolately lobed, serrated, upper leaves linear-lanceolate, entire ; flowers alternate, pink, mottled, slightly hairy, large (4·3×1·3 cm.); capsules solitary, slightly hairy (2·5×1·1 cm.), 8 loculi ; seeds drab ; life-period 80 days.

Type 17.—Plants short (86 cm.), medium branched; leaves large, dark green, petioles red, lower leaves broadly ovate, serrated, middle leaves lobed, much serrated, upper leaves lanceolate, entire ; flowers opposite, light pink, mottled, very hairy (4×1 cm.), capsules 3 per axil, hairy (3×0·6 cm.), 4 loculi, seeds drab ; life-period 83 days.

Type 18.—Plants medium height (106 cm.), branched; leaves medium size, light green, petioles red, lower leaves ovate, entire, middle leaves lobed, serrated, upper leaves linear-lanceolate, slightly serrated; flowers alternate, pink, faintly mottled, hairy (4×1 cm.); capsules solitary, hairy (2·9×0·6 cm.), 4 loculi, seeds brown ; life-period 92 days.

Type 19.—Plants short (96 cm.), branched; leaves large, dark green, petioles red, lower leaves narrowly ovate, entire, middle leaves lobed, serrated, upper leaves linear-lanceolate, entire ; flowers opposite, light pink, not mottled, slightly hairy (4·1×0·8 cm.); capsules solitary, slightly hairy, long (3·2×0·6 cm.), 4 loculi ; seeds brown ; life-period 80-90 days. (*Hnanni* 25-160).

Type 20.—Plants short (88 cm.), branched; leaves small, dark green, petioles green, lower leaves ovate, entire, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire ; flowers alternate, white, not mottled, hairy (3·9×1 cm.); capsules solitary, hairy, short (2·5×1 cm.), 6 to 8 loculi, seeds brown ; life-period 85 days.

Type 21.—Plants medium height (107 cm.), branched ; leaves small, light green, petioles red, lower leaves broadly ovate, entire, middle leaves lobed trifoliolately,

slightly serrated, upper leaves linear-lanceolate, entire; flowers alternate, white, not mottled, slightly hairy (4×1 cm.); capsules solitary, slightly hairy, very short (2·4×0·8 cm.), 6 to 8 loculi, seeds brown; life-period 92 days.

Type 22.—Plants short (70 cm.), unbranched; leaves small, dark green, petioles red, lower leaves broadly ovate, entire, middle leaves lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, light pink, not mottled, slightly hairy (3·8×1·1 cm.); capsules solitary, slightly hairy, very short (2·2×1·1 cm.), 8 loculi, seeds black; life-period 80 days.

Type 23.—Plants short (90 cm.), unbranched; leaves medium size, dark green, petioles red, lower leaves ovate, entire, middle leaves lobed deeply, serrated, upper leaves linear-lanceolate, entire; flowers opposite, pink, mottled, very hairy (3·8×1 cm.); capsules 3 per axil, hairy, short (2·5 ×1·1 cm.), 6 to 8 loculi, seeds black; life-period 83 days.

Type 24.—Plants tall (127 cm.), unbranched; leaves large, dark green, petioles red, lower leaves ovate, serrated, middle leaves ovate, lobed deeply, serrated, upper leaves linear-lanceolate, slightly serrated; flowers alternate, pink, not mottled, hairy (4·3×1·1 cm.); capsules solitary, hairy (2·8×0·6 cm.), 4 loculi, seeds white; life-period 82 days. (*Thadunbyu* 26-136).

Type 25.—Plants tall (132 cm.), unbranched; leaves large, dark green, petioles red, lower leaves ovate, slightly serrated, middle leaves ovate, lobed, serrated, upper leaves linear-lanceolate, entire; flowers alternate, pink, not mottled, slightly hairy (4·1×1 cm.); capsules solitary, slightly hairy (2·9×0·6 cm.), 4 loculi, seeds white; life-period 80 days.

Type 26.—Plants short (90 cm.), unbranched; leaves medium size, light green, petioles light purple, lower leaves narrowly ovate, entire, middle leaves ovate, lobed, serrated, upper leaves linear-lanceolate, entire; flowers alternate, deep pink, mottled, hairy (4·1×1·1 cm.); capsules solitary, hairy, short (2·5×1 cm.), 8 loculi, seeds white; life-period 80 days.

Type 27.—Plants tall (132 cm.), unbranched, leaves large, dark green, petioles red, lower leaves ovate, slightly serrated, middle leaves ovate, lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, white or pink, not mottled, slightly hairy (4·3×1 cm.); capsules solitary, slightly hairy, short (2·8×1 cm.), 8 loculi, seeds white; life-period 80 days. (*Hnangyi* 25-187.)

Type 28.—Plants tall (130 cm.), unbranched. leaves large, dark green, petioles red, lower leaves narrowly ovate, much serrated, middle leaves lobed deeply, serrated, upper leaves linear-lanceolate, entire; flowers opposite, deep pink, mottled, slightly hairy, large (4·4×1 cm.); capsules 3 per axil, hairy, long (3·2×0·8 cm.), 4 loculi, seeds white; life-period 94 days. (*Taingdaung* 25-181.)

Type 29.—Plants short (96 cm.), unbranched; leaves small, greyish green, petioles red, lower leaves ovate, serrated, middle leaves narrowly ovate, lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers alternate, pink, not mottled, slightly hairy (4·1×1 cm.); capsules 2 to 3 per axil, slightly hairy (2·9×0·8 cm.), 4 loculi, seeds white; life-period 82 days.

Type 30.—Plants short (91 cm.), unbranched; leaves small, dark green, petioles red, lower leaves ovate, serrated, middle leaves ovate, lobed trifoliolately, serrated, upper leaves linear-lanceolate, entire; flowers opposite, deep pink, mottled, very hairy (3·8×1 cm.); capsules 2 to 3 per axil, the lowest pair often solitary, hairy, (2·5×1 cm.), 8 loculi, with a few lateral capsules of 4 loculi; seeds white; life-period 83 days.

Type 31.—Plants short (90 cm.), unbranched; leaves small, light green, petioles red, lower leaves ovate, entire, middle leaves narrowly ovate, not lobed, serrated, upper leaves linear-lanceolate, entire; flowers opposite, pink, not mottled, very hairy (3·8×1 cm.); capsules solitary, hairy (2·8×0·6 cm.), 4 loculi, seeds drab; life-period 80 days.

Type 32.—Plants tall (122 cm.), unbranched; leaves large, dark green, petioles red, lower leaves narrowly ovate, slightly serrated, middle leaves ovate, lobed, serrated, upper leaves linear-lanceolate, entire; flowers alternate, deep pink, faintly mottled, slightly hairy (4·1×1·1 cm.); capsules solitary, slightly hairy, short (2·5×1·1 cm.), 8 loculi, seeds drab; life-period 82 days.

Type 33.—Plants short (76 cm.), unbranched; leaves large, dark green, petioles light green, lower leaves broadly ovate, middle leaves ovate, lobed, much serrated, upper leaves lanceolate, entire; flowers opposite, light pink, mottled, very hairy (3·8×1 cm.); capsules 3 per axil, very hairy (2·9×0·8 cm.), 4 loculi, seeds drab; life-period 78 days.

Type 34.—Plants short (76 cm.), unbranched; leaves medium size, petioles red, lower leaves broadly ovate, slightly serrated, middle leaves broadly ovate, lobed, serrated, upper leaves lanceolate, entire; flowers opposite, pink, not mottled, very hairy (4·1×1 cm.); capsules 3 per axil, very hairy (2·7×0·8 cm.), 4 loculi, seeds brown; life-period 89 days. (*Boktaung* 25-225.)

Late sesamum.

The late sesamums amount to about twent-five per cent. of the total area under sesamum in Burma. They are grown all over the plains and form the principal class in Lower Burma.

Fewer late types have been studied than early. They are not so important and do not appear to be so numerous. The same characters are used for classification.

KEY TO THE TYPES OF BURMESE 'LATE' SESAMUM.

A—Plants branched.
I—Seeds black.
(1) Number of capsules—one per axil.
α—Loculi—four per capsule.
a. Capsules hairy . . . . . Type 1
b. Capsules slightly hairy. . . . . Type 2
β—Loculi—more than four per capsule.
a. Capsules hairy . . . . . Type 3
b. Capsules slightly hairy . . . . . Type 4
(2) Number of capsules—more than one per axil.
α—Loculi—four per capsule.
a. Capsules hairy. . . . . Type 5
II—Seeds white.
(1) Number of capsules—one per axil.
α—Loculi—four per capsule.
a. Capsules hairy . . . . . Type 6
b. Capsules—slightly hairy . . . . Type 7
β—Loculi—more than four per capsule.
b. Capsules slightly hairy . . . . . Type 8
III—Seeds drab.
(1) Number of capsules—one per axil.
α—Loculi—four per capsule.
a. Capsules hairy . . . . . Type 9
β—Loculi—more than four per capsule.
a. Capsules hairy . . . . . Type 10
IV—Seeds brown.
(1) Number of capsules—one per axil.
α—Loculi—four per capsule.
a. Capsules hairy . . . . . Type 11
b. Capsules slightly hairy . . . . . Type 12
B—Unbranched.
II—Seeds white.
(2) Number of capsules—more than one per axil.
α—Loculi—four per capsule.
a. Capsules hairy . . . . . Type 13
b. Capsules slightly hairy . . . . . Type 14
β—Loculi—more than four per capsule.
a. Capsules hairy . . . . . Type 15

DESCRIPTION OF TYPES OF LATE SESAMUM.

Type 1.—Plants medium height (106 cm.), branched ; leaves light green petioles dull red, lower leaves ovate, serrated, middle leaves lobed trifoliolately, serrated and upper leaves linear-lanceolate, entire ; flowers opposite, deep pink, not mottled, very hairy ; capsules solitary, hairy, short, 2·4×0·9 cm., 4 loculi ; seeds black ; life-period 105 days.

Type 2.—Plants short (76 to 100 cm.), branched ; leaves light green, petioles dull red, lower leaves ovate, serrated, middle leaves trifoliolately lobed, serrated and upper leaves linear-lanceolate, entire ; flowers alternate, deep pink, not mottled, hairy ; capsules solitary, slightly hairy, short, 2·3×0·9 cm., 4 loculi ; seeds black ; life-period 105 days.

Type 3.—Plants short (86 cm.), branched ; leaves dark green, petioles red, lower leaves ovate, entire, middle leaves ovate to lanceolate, entire and upper leaves linear-lanceolate, entire ; flowers alternate, pink, not mottled, hairy ; capsules solitary, hairy, short, 2×1 cm., more than 4 loculi ; seeds black ; life-period 105 days.

Type 4.—Plants short (76 cm.), branched ; leaves light green, petioles dull red, lower leaves ovate, slightly serrated, middle leaves trifoliolately lobed, serrated and upper leaves linear-lanceolate, entire ; flowers alternate, deep pink, not mottled, hairy ; capsules solitary, slightly hairy, short, 2·1×1 cm., more than 4 loculi ; seeds black ; life-period 105 days.

Type 5.—Plants short (86 cm.), branched ; leaves light green, petioles dull red, lower leaves ovate, entire, middle leaves ovate to lanceolate, slightly serrated and upper leaves linear-lanceolate, entire ; flowers opposite and alternate, deep pink, deeply mottled, hairy ; capsules more than one per axil, hairy, short, 1·8×0·8 cm., 4 loculi ; seeds black ; life-period 105 days.

Type 6.—Plants very short (60 cm.), semi-branched ; leaves light green, hairy, petioles green, lower leaves ovate, serrated, middle leaves trifoliolately lobed, serrated and upper leaves linear-lanceolate, entire ; flowers alternate, small, pink, not mottled, very hairy ; capsules solitary, very hairy, 3×0·9 cm., 4 loculi ; seeds white ; life-period 76 days.

Type 7.—Plants very short (60 cm.), branched ; leaves light green, petioles dull red, lower leaves ovate, serrated ; middle leaves lanceolate, serrated and upper leaves linear-lanceolate, entire ; flowers alternate, pink, not mottled, hairy ; capsules solitary, slightly hairy, short, 2·6×0·6 cm., 4 loculi ; seeds small, white ; life-period 71 days.

Type 8.—Plants very short (60 cm.), branched ; leaves light green, petioles dull red, lower leaves ovate, serrated, middle leaves lanceolate, serrated and

upper leaves linear-lanceolate, entire; flowers opposite or alternate, pink, not mottled, hairy; capsules solitary, slightly hairy, 2·5 to 3×1 cm., more than 4 loculi per capsule; seeds small, white; life-period 71 days.

Type 9.—Plants short (86 cm.), branched; leaves light green, petioles dull red, lower leaves ovate, entire, middle leaves ovate to lanceolate, slightly serrated and upper leaves linear-lanceolate, entire; flowers alternate, deep pink, deeply mottled, hairy; capsules solitary, hairy, very short, 1·8×0·8 cm., 4 loculi; seeds medium size, drab; life-period 105 days.

Type 10.—Plants short (86 cm.), branched; leaves light green, petioles dull red, lower leaves ovate, entire, middle leaves ovate to lanceolate, slightly serrated and upper leaves linear-lanceolate, entire; flowers alternate, deep pink, deeply mottled, hairy; capsules solitary, hairy, very short, 1·8×1·0 cm., more than 4 loculi per capsule; seeds drab; life-period 105 days.

Type 11.—Plants tall (152 to 172 cm.), branched; leaves light green, petioles green to dull red, lower leaves ovate, slightly serrated, middle leaves trifoliolately lobed, serrated and upper leaves linear-lanceolate, entire; flowers alternate, deep pink, not mottled, very hairy; capsules solitary, hairy, 2·5 to 2·7×1·0 cm., 4 loculi; seeds brown; life-period 110 days.

Type 12.—Plants very tall (172 to 182 cm.), much branched; leaves dark green, large, petioles dull red, lower leaves ovate, middle leaves lobed deeply to trifoliolately, serrated and upper leaves linear-lanceolate, entire; flowers deep pink, faintly mottled, hairy; capsules solitary, slightly hairy, 3·0×0·9 cm., 4 loculi; seeds brown; life-period 107 days.

Type 13.—Plants short (90 cm.), unbranched; leaves light green, petioles green, lower leaves ovate, entire, middle leaves ovate to lanceolate, entire and upper leaves linear-lanceolate, entire; flowers opposite, deep pink, mottled, very hairy; capsules more than one per axil (in lower axils solitary), very hairy, 2·8×0·8 cm., 4 loculi; seeds large, white; life-period 91 days.

Type 14.—Plants tall (120 to 130 cm.), unbranched; leaves dark green, petioles dull red, lower leaves ovate, entire, middle leaves ovate to lanceolate, entire and upper leaves linear-lanceolate, entire; flowers alternate, pink, not mottled, hairy; capsules solitary at the lowest two nodes but more than one per axil above, slightly hairy, short, 2·1×1·0 cm., 4 loculi; seeds large, white; life-period 76 days.

Type 15.—Plants short (90 cm.), unbranched; leaves and petioles light green, lower leaves ovate, entire, middle leaves ovate to lanceolate, entire and upper leaves linear-lanceolate, entire; flowers opposite, deep pink, mottled, very hairy; capsules solitary at the lowest nodes and more than one per axil above, very hairy, more than 4 loculi; seeds white; life-period 91 days.

Summary.

1. The distinction between monsoon and winter sesamum varieties is emphasised and the result of crosses between these two types recorded. The crosses indicate that the late (winter) character is dominant and that one principal gene is concerned.

2. The occurrence of association between various characters was tested by means of four-fold tables and the results are recorded.

3. Classification is based on branching habit, seed-coat colour, number of capsules per axil, number of loculi per capsule and hairiness of the capsule.

4. Thirty-four types of early (monsoon) sesamum and fifteen types of late (winter) sesamum are described.

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Appendix.

The fourfold tables from which the values of χ^2 and P in Table III were calculated are given below.

1. Branching and number of capsules per axil.

	Branched		Unbranched	
	One capsule	Over one capsule	One capsule	Over one capsule
Observed $(m+x)$	153	21	36	17
Expected (m)	144·87	29·13	44·13	8·87
$\frac{x}{m}$	0·4562	2·2656	1·4955	7·4517

$\chi^2 = 11{\cdot}6690.$ $P = {\cdot}0086.$

2. Branching and seed-coat colour.

	Branched		Unbranched	
	White	Coloured	White	Coloured
Observed $(m+x)$	104	69	46	7
Expected (m)	114·82	58·18	35·18	17·82
$\frac{x^2}{m}$	1·019	2·012	3·040	6·569

$\chi^2=12·640.$ $P=·0055.$

3. Branching and number of loculi per capsule.

	Branched		Unbranched	
	4 loculi	Over 4 loculi	4 loculi	Over 4 loculi
Observed $(m+x)$	94	79	31	22
Expected (m)	95·68	77·32	29·32	23·68
$\frac{x^2}{m}$	0·0295	0·0365	0·0963	0·1192

$\chi^2=0·2815.$ $P=·9507.$

4. Seed-coat colour and number of loculi.

	White		Coloured	
	4 loculi	Over 4 loculi	4 loculi	Over 4 loculi
Observed $(m+x)$	76	74	49	27
Expected (m)	82·964	67·036	42·026	33·964
$\frac{x^2}{m}$	0·584	0·723	1·153	1·428

$\chi^2=3·888.$ $P=·2736.$

5. Number of capsules per axil and number of loculi per capsule.

	One capsule		Over one capsule	
	4 loculi	Over 4 loculi	4 loculi	Over 4 loculi
Observed $(m+x)$	95	93	30	8
Expected (m)	103·98	84·02	21·02	16·98
$\frac{x^2}{m}$	0·775	0·959	3·836	4·749

$\chi^2=10·319.$ $P=·0172.$

6. Seed-coat colour and number of capsule.

	White		Coloured	
	One capsule	Over one capsule	One capsule	Over one capsule
Observed $(m+x)$	116	34	72	4
Expected (m)	124·77	25·23	63·23	12·77
$\frac{x^2}{m}$	0·6164	3·0485	1·2164	6·0229

$\chi^2=10·9042.$ $P=·0122.$

7. Branchiag and sepaloidy.

	Branched		Unbranched	
	High susceptibility	Low susceptibility	High susceptibility	Low susceptibility
Observed $(m+x)$	63	101	39	17
Expected (m)	76·04	87·96	25·96	30·04
$\frac{x^2}{m}$	2·236	1·9331	6·5501	5·6604

$\chi^2=16·3796.$ $P=·0009.$

PHYSIOLOGY OF *CERCOSPORA DOLICHI* E. AND E.

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(With Plates XXXII-XXXIV and seven text-figures)

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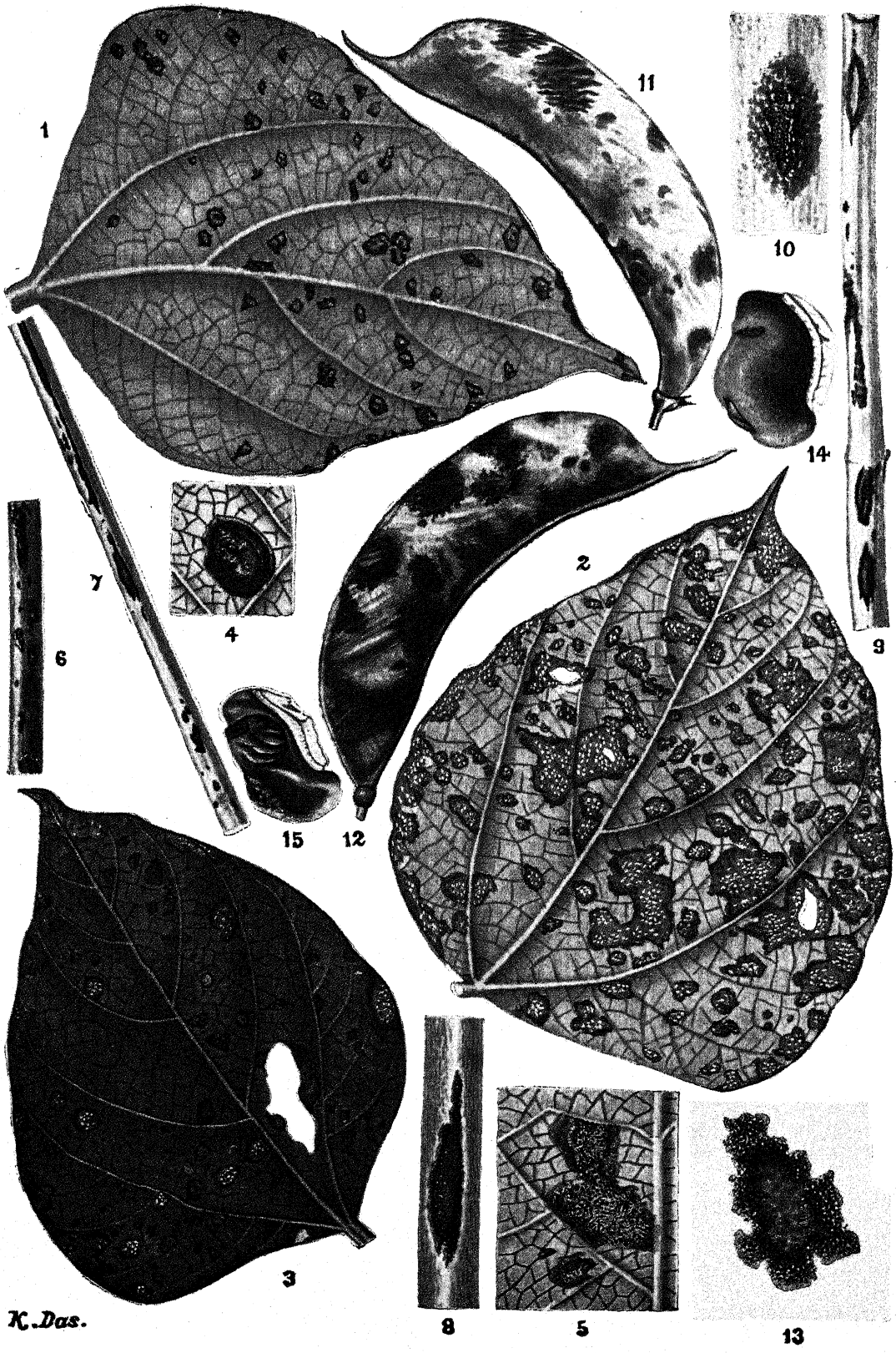


(For explanation see p, 528).

I.—Introduction.

The present investigation deals with the nature and etiology of *Cercospora dolichi* E. and E. parasitic on *Dolichos Lablab* Linn., its physiological responses to various environmental conditions, infectivity and variations. These studies throw light on the role of external factors on the nature and spread of the disease.

II.—Morphology of the organism in nature.

(*a*) *Symptoms*.

Leaves.—The spots formed on the leaves are in the beginning circular, 2-10 mm. in diameter, but later become more or less irregular on account of the coalescence of two or more such spots and these irregular spots are often as big as 2-3 cm. in diameter. They are reddish in the beginning but later on show differentiation of colour into distinct zones. The central raised portion is of bluish grey colour and bears conidiophores and conidia and is surrounded by a reddish zone which abruptly merges into the green of the leaf (Plate XXXII, figs. 1-5). The lesions are more pronounced on the under surface and are limited by the veins. In advanced stages the spots are also found on the upper surface when the whole leaf curls and eventually falls off.

Petioles.—The young lesions on the petiole appear as slightly darkened areas along its length and the spots are reddish in the beginning, but gradually grow dark in the centre surrounded by a slightly purplish border as in the leaves and the margin is irregular (Plate XXXII, figs. 6, 7 and 8.).

Stems.—The stem as a rule is much less infected than the leaves and the pods. In shape and colour the lesions resemble those on the petioles as shown in Plate XXXII, figs. 9 and 10. The whole stem eventually dries and the plant droops down.

Pods.—The lesions on the pods are numerous. They are more or less circular and measure from 1 to 8 mm. in diameter. Irregular spots are not uncommon. In early stages the spots are of scarlet colour in the centre with an orange coloured border, but in advanced stages the scarlet centre is usually surrounded by a greyish black zone bearing conidiophores and conidia (Plate XXXII, figs. 11, 12 and 13). In the end the whole pod becomes blackish and is destroyed by the parasite.

Seeds are also affected, the infected portions become at first brownish yellow with irregular depressions and the whole seed is gradually killed (Plate XXXII, figs. 14 and 15). The percentage of infection in seeds was found to be nineteen.

(b) *Morbid anatomy.*

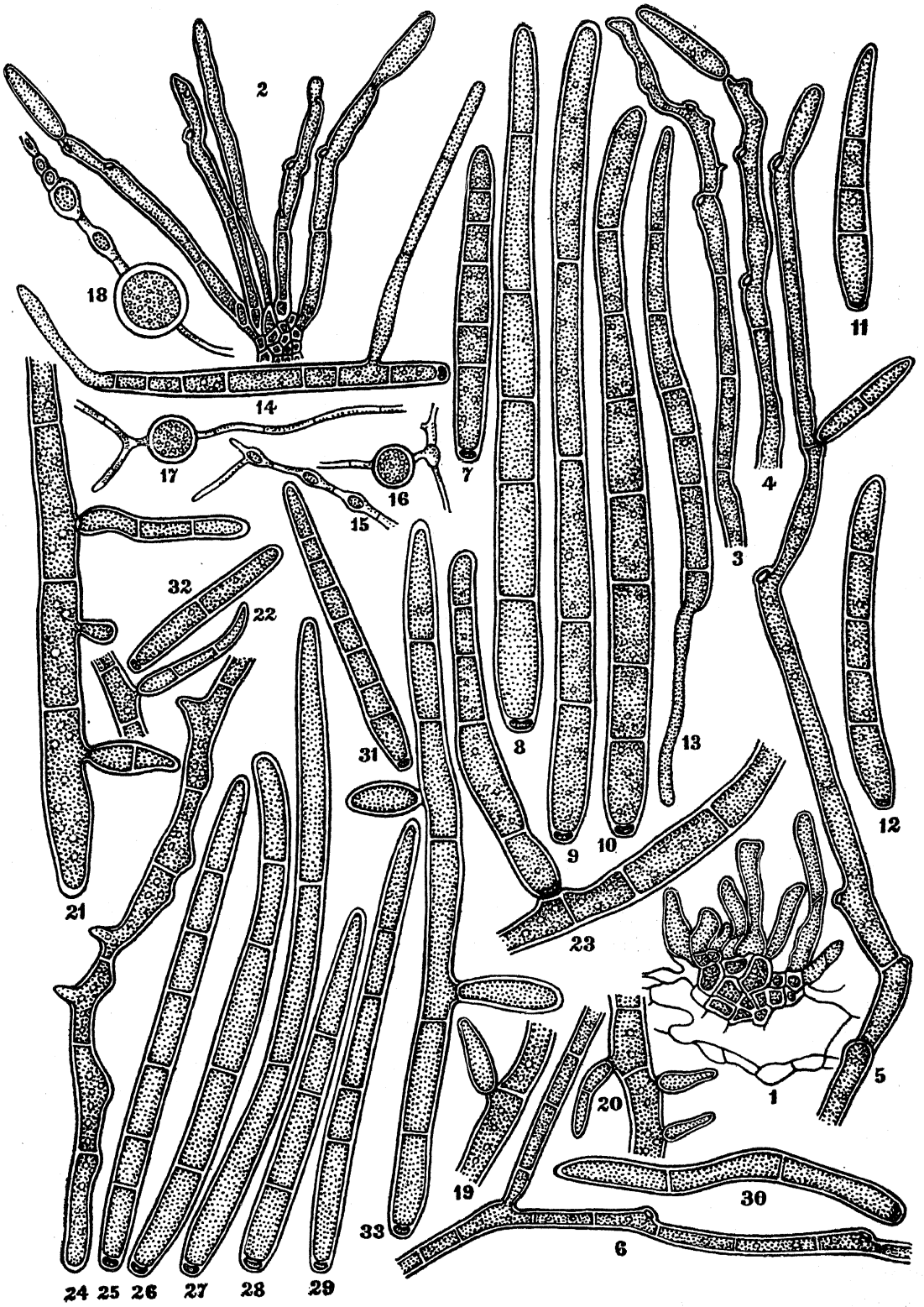
The mycelium of the fungus within the host tissue is composed of irregularly septate, thick-walled light brown hyphæ, varying from 1·5 to 3·5 μ in diameter, but at times upto 6 μ in the case of hyphæ forming stromata from which conidiophores arise. Younger hyphæ are thin-walled and sparsely septate while the older ones are brown, thick-walled, tortuous with septa at short intervals and sparsed with granular protoplasm and numerous oil globules. Mycelium is usually intercellular, but in old disintegrated tissues it also becomes intracellular with knob-shaped haustoria. Hyphæ collect in the air spaces underneath the stomata and form stromatic masses 30 μ-60 μ in diameter (Plate XXXIII, figs. 1 and 2) which often project out of the stomata. Disintegration of the cells of palisade and spongy porenchyma takes place. The cells are killed in advance of the hyphæ. Guard cells of the stomata are thrown apart as the bundle of conidiophores protrudes out of the stomata. The first cytological change in injured cells as observed in the margin of sectioned lesions is the slight disintegration of the chloroplasts accompanied by changes in staining reaction, the infected tissue staining deeply. This is followed by the disintegration of the protoplasts and then by the complete collapse of the cells. Disintegration is most evident where stroma are formed.

(c) *Conidiophores.*

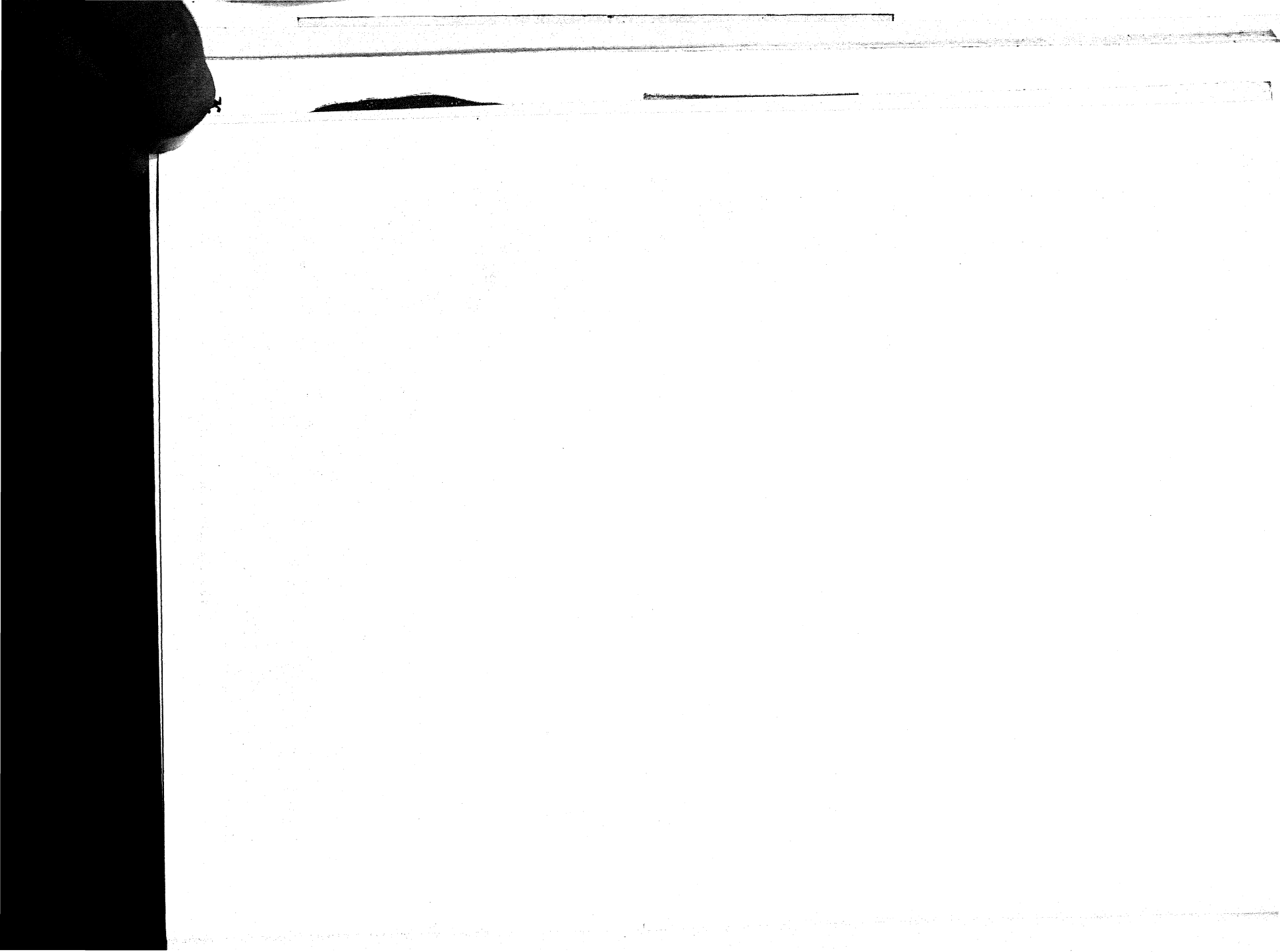
These are formed from stromatic masses either inside the tissue or on the central dead portion of the spot and are more on the under surface. When they are formed from the stromatic masses under the epidermis, they emerge through stomata in groups of two or more. These are of knee-joint appearance on account of the position of conidia and measure 90·8-527 μ × 5·1-6·8 μ; septa from 0 to 15 (Plate XXXIII, figs. 2-5). Sometimes branched conidiophores are also found (Plate XXXIII, fig. 6). Conidiophores are long, multi-septate, yellowish brown while young but dark brown in advanced stages. They readily germinate in tap water sending out germ tubes from both ends as well as from sides near the septa.

(d) *Conidia.*

These are borne on the conidiophores in aeropetal succession leaving a scar when they get detached. They are sub-hyaline to light yellowish, multi-septate (1-15), acicular to acicular abclavate and in size vary from 17·0-221·0 μ × 3·4-6·8 μ, the average being 63 μ (Plate XXXIII, figs. 7-12). The distal end of the conidium has a circular hilum with a slight depression in the centre marking the position of the attachment to conidiophore. The conidia taper gradually from the proximal end towards the distal end, the former being much wider than the latter.



(*For explanation see p. 529.*)



III.—Cultural characteristics.

Several single spore isolations of the fungus were obtained from various parts of infected plants and all proved to be identical. Re-isolations from artificially inoculated leaves of *Dolichos Lablab* were also made and agreed with the original isolations.

The various physiological responses of the fungus were studied. The fungus was cultivated on a large number of artificial media, both solid and liquid. The details of these studies are given below.

(A) Macroscopic characters.

(*a*) *Growth on different media.*

(*i*) *Nature of growth.*—The fungus grows well on all media employed except on Browns' synthetic agar in which staling takes place. On Coons' agar, prune juice agar and *Dolichos Lablab* leaf decoction agar growth is mostly submerged throughout the colony with very sparse aerial mycelium along the edge. On richer media such as Doxs' agar, Richards' solution agar, Hopkins' agar, both aerial and submerged growths are copious and the colonies are uneven and crinkled. Above optimum temperature the growth of the fungus in all these media is poor and the aerial mycelium becomes felty. In very old cultures sclerotial bodies are formed on the edge of Petri dishes and also on the upper surface of the slants.

(*ii*) *Nature and colour of the aerial mycelium and colour of the substratum.*—The amount of aerial mycelium increases with the rise in temperature upto the optimum for growth. Above optimum temperature it becomes hard and felty and there is total disappearance of the loose type of aerial mycelium at temperatures above 30°C. It goes on increasing with the rise in relative humidity upto the optimum for growth, that is, 78·7 beyond which there is a decrease. It is greater on thickly poured plates than on thinly poured ones. At concentrations above normal ranging upto 10 *N* on Richards' solution agar and Coons' agar the aerial mycelium becomes felty in structure and abundant while at concentrations below *N*, from *N*-*N*/100 it becomes loose in texture, woolly or cottony and sparse. On media like Richards' solution agar, the amount of aerial mycelium is greater than on poor media like Browns' synthetic agar, plain agar, Beyrincks' agar and Cornmeal agar. Moreover, on rich media this fungus gives rise to a felty type of aerial mycelium. It is greater in alternate light and darkness than in complete darkness. A study of the fungus on 5 per cent. sucrose, glucose, lactose and maltose showed total suppression of aerial mycelium on the first two, best growth

on maltose agar and least on lactose agar. A regular margin of the colony was observed in all media tried except in the case of Richards' solution agar and Doxs' agar where the margin showed a tendency to form lobes. The colour of the aerial mycelium is remarkably affected with the nature of the media. The other factors such as temperature, humidity, light and darkness, the concentration of the medium, etc., affect the colour of the aerial mycelium to a lesser extent. The details are given below :—

TABLE I.

Nature and colour of aerial mycelium, and the colour of the substratum of C. dolichi *E. and E. on different media at 25°C.*

Media	Cultural characters
Coons' agar	Growth copious and cottony, light purplish, sparse cobwebby, light pale grey green on the edge. Substratum dark olive green.
Oatmeal agar	Abundant woolly, light purplish tinted white, scanty of light forget-me-not blue on the margin. Substratum, light greyish indigo in the centre, edge light sky blue.
Richards' solution agar . .	Abundant felty, light sky blue colour in the centre, woolly and pale rose pink on the edge. Radial convolutions very common. Cobwebby structure in the middle portion of the colony appears from which clear water oozes out. Substratum, centre dark artichoke green surrounded by light greyish indigo zone, extreme edge light yellow.
Doxs' agar	Abundant slightly felty, brownish grey, edge light lilacy white. Convolutions and cobwebby structure similar to that on Richards, solution agar. Substratum, dark grey green in the centre, edge dark sheet blue.
Brown's starch medium . .	Abundant loose cottony, light lilacy white, on edge sparse and low of dull white. Substratum, light greyish indigo in the centre, edge dark olive green.
Dolichos Lablab leaf decoction agar	Copious cottony, light lilacy white with patches of light otter brown, sparse and low on the edge. Substratum, dark putty colour, edge light yellow.
Hopkins' agar	Abundant, woolly, light lilacy white, sparse on the edge. Substratum light cinnamon in the centre, then surrounded by a zone of dark sap green, edge light stone colour. Suppression of aerial mycelium with age.

The fungus was also studied on several other media like sucrose agar, lactose agar, glucose agar and maltose agar.

TABLE II.

Nature and colour of aerial mycelium, and the colour of the substratum of C. dolichi *E. and E. at different temperatures on different media.*

Temperature	Coons' agar	Richards' solution agar	Oatmeal agar
10°C.	Sparse cottony, light succony blue, absent on the edge. Substratum, dark greyish indigo, edge light stone colour.	Abundant, woolly, dark slate grey, on edge sparse light lilacy white. Substratum, light buff, edge light putty colour.	Profuse cottony, light eucalyptus green, sparse sky blue on edge. Substratum, dark titmouse blue, edge light stone colour.
20°C.	Less copious than at 25°C., loose cottony, light purplish tinted white, very sparse eucalyptus green on edge. Substratum, olive green.	Abundant, felty titmouse blue, woolly, yellowish white on the edge. Substratum, dark sea green, then zone of light greyish indigo, extreme edge light sulphury white.	Abundant, woolly, light forget-me-not blue, less abundant on the edge. Substratum, light blue slate.
25°C.	Abundant, loose cottony, light purplish tinted white, sparse, cobwebby, pale grey green on edge. Substratum, dark olive green.	Very abundant felty light sky blue, woolly pale rose pink on the edge, Substratum, light artichoke green, edge greenish indigo to light yellow.	Profuse, woolly, light purplish tinted white, sparse, forget-me-not blue on the edge. Substratum, light greyish indigo ; edge light sky blue.
27·5°C.	Abundant loose cottony, light lilacy white, sparse, light plumbago blue on the edge. Substratum, light cinnamon in the centre; edge light Van Dyck brown to blood red brown.	Abundant, felty, pale rose pink, sparse light cobalt blue to light lilacy on the edge. Substratum, dark artichoke green, edge light grey green to light yellow.	Abundant, woolly, light lilacy white suppressed on the edge. Substratum, dark titmouse blue, edge pale grey green.
30°C.	Less abundant, woolly, light lilacy white, absent on the edge. Substratum, dark flesh colour, edge dark ivory green to blue greenish grey.	Abundant, hard felty, light purplish tinted white. Substratum, dark drab green, edge titmouse blue to light yellow.	Abundant, slightly woolly light lilacy white. Substratum, bright greenish grey; edge light sky blue.
32·5°C.	Abundant, slightly felty light lilacy white. Substratum, dark olive green, edge dark fawn.	Abundant, very felty, light fleshy white. Substratum, dark verdigris, edge light severe blue, slight sky blue.	Abundant, slightly felty, light rosy flush, edge light lilacy white. Substratum, light blue slate.
35°C.	Copious, hard felty, light mauve rose. Substratum, dark olive green ; edge dark fawn to light eucalyptus green.	Abundant, hard felty, light greenish white, suppressed on the edge. Substratum, light coppery yellow ; edge dark grey green to light maize yellow.	Abundant, hard felty, light peach blossom, low on the edge. Substratum, light bluish sea green.
37·5°C.	No growth . . .	Abundant, very hard and felty, pure white. Substratum, dark maize yellow.	No growth.

(*b*) *Depth of medium.*

In order to determine the influence of the depth of the medium Petri dishes of equal size were supplied with 12, 25 and 50 c.c. of Coons' agar and oatmeal agar.

Triplicate plates were used for each series. The inoculated plates were incubated at 27·5 C. The diameter of the colonies was measured after ten and twenty days. The details are given in Table III.

TABLE III.

Influence of depth of medium on linear growth of C. dolichi *E. and E.*

Media	Amount of media	Days	
		10	20
	c.c.	mm.	mm.
Coons' agar. . .	12	41·6	60·6
	25	45·6	67·0
	50	46·0	75·0
Oatmeal agar . .	12	40·0	66·0
	25	40·5	70·5
	50	43·0	80·6

It will be seen from Table III that the linear growth increases with the increase in the depth and amount of nutrient. Similarly the colour of the colony becomes deeper. The growth after 20 days in plates supplied with 50 c.c. was profuse and matted. Experiments with slanted plates of media showed that the growth was greater on the thicker side of the slant than on the thinner. Similar results were obtained when the fungus was grown on liquid cultures. Although Coons' and Larmer [1930] found in the case of *C. beticola* that the depth of media had very little influence on growth, the observations made by the author, however, agree with those made by Mitra [1931, 1], in the case of *Helminthosporium*. It therefore seems that in carrying out determination of growth rate under different environmental conditions, the depth of the medium should be uniform.

(*c*) *Light.*

To study the effect of alternate light and darkness, inoculated plates of Coons' agar were placed in front of a window at room temperature. Alongside of them, cultures to be kept in continuous darkness were put inside big blackened cover dishes after wrapping them with black paper. Cultures to be kept in

continuous light were placed in front of a 100-watt electric bulb in a dark room alongside of which were placed plates in complete darkness. The diameters of the different colonies were measured after 13 and 26 days' intervals.

TABLE IV.

Effect of alternate light and darkness, continuous light and continuous darkness on Coons' agar on growth of C. dolichi *E. and E.*

——	13 days	Ratio D/L	26 days	Ratio D/L
	mm.		mm.	
Alternate light and darkness	32	88	44·3	81·1
Continuous darkness	28·3		36	
Continuous light	21·5	114·4	30·3	120·3
Continuous darkness	24·0		36·5	

It will be seen from Table IV that the linear rate of growth is greater in alternate light and darkness than in complete darkness and is greater in complete darkness than in continuous light, the retarding effect of continuous darkness and continuous light becoming more pronounced with time.

(*d*) *Humidity.*

A study of the growth rate of the fungus under different relative humidities was made in accordance with the method of Stevens [1916]. Sulphuric acid of varying strength was poured into five glass dishes of uniform size and each dish gave a certain definite relative humidity, *viz.*, 47, 68, 70·4, 78·7, 92·3 per cent. To represent approximately 100 per cent. humidity, 250 c.c. of sterilized distilled water was poured in a sixth dish. Plates inoculated in the centre were fixed on sterilized glass sheets with a mixture of gelatine and corrosive sublimate in order to avoid contamination and were inverted after removing the lid on big glass dishes containing various percentages of sulphuric acid, and finally sealed all along the side with vaseline. In this way all the inoculated plates were exposed to various relative humidities created by sulphuric acid and these dishes were kept at room temperature. The experiment was repeated with two media, *i.e.*, Doxs' agar and Dolichos leaf decoction agar. Measurements were made at intervals of four days up to sixteen days and the data obtained are plotted out in Fig. 1. It will be observed therefrom that the fungus tolerates a wide range of humidity and the optimum lies at about 78·7 per cent.

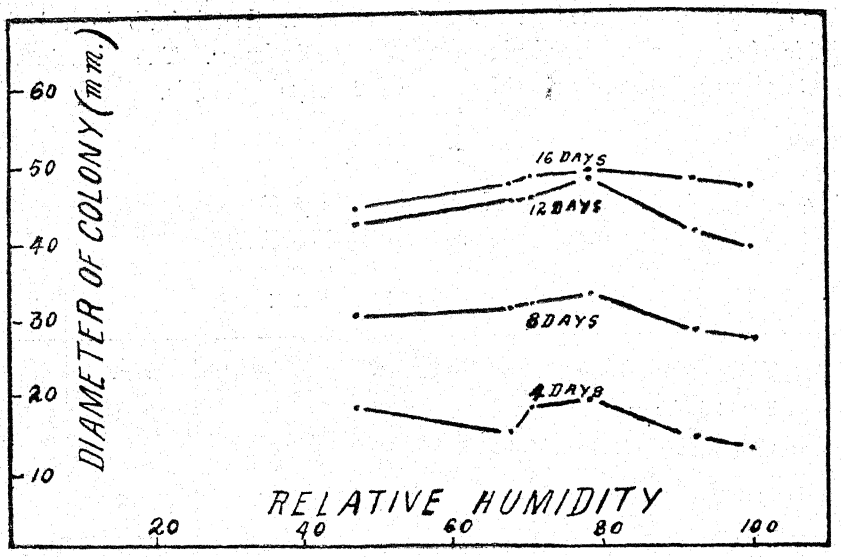
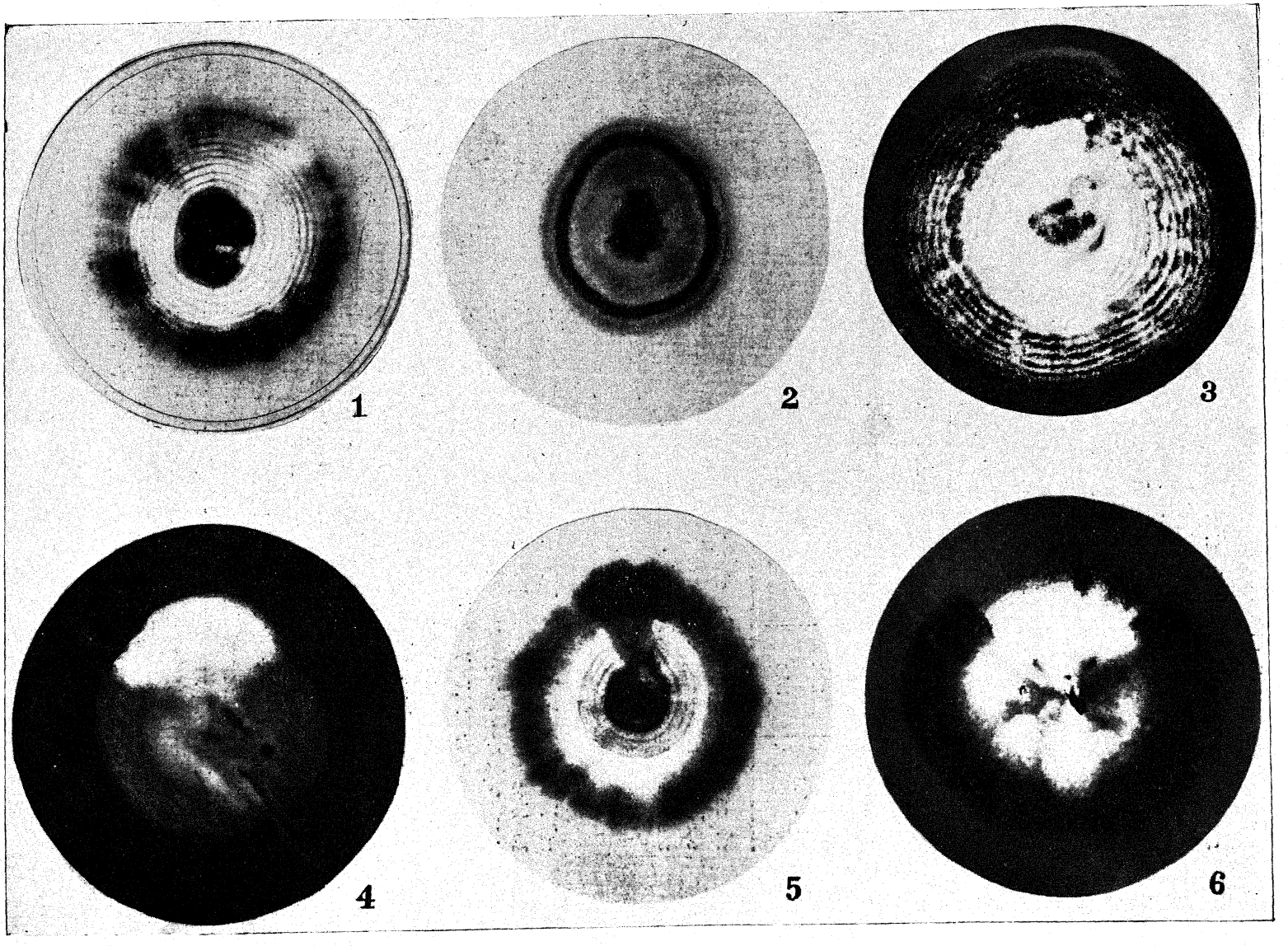


Fig. 1.—Growth of *C. dolichi* in 4, 8, 12 and 16 days of different relative humidities.

(*e*) *Zonation.*

Zonation in *C. dolichi* E. and E., is due to differential rate of linear growth of the submerged mycelium as found by Coons and Larmer [1930]. Alternating light and darkness plays the most important part in the formation of zones; other factors such as fluctuating temperature and the dilution of media are of less importance.

(*i*) *Alternating light and darkness.*—Some inoculated Petri dishes were placed near a window exposed to daylight together with others wrapped up to avoid light. Petri dishes were also kept in continuous light (light furnished by a 100-watt electric bulb) in a dark room together with some dishes wrapped up in black paper. It was found that zonation was prominent in alternating day and night, and altogether absent in continuous light or complete darkness (Plate XXXIV, fig. 1). Further it was observed that it was more prominent in plates kept in an inverted position. Zonation was further noticed to be more prominent on certain media and absent altogether on others. The data obtained are given in Table V. A study of the effect on zonation of different periods of exposure to light was carried out. Inoculated plates of Coons' synthetic agar, *N*/5 Richards' solution agar, and oatmeal agar, both thickly and thinly poured, were placed in an incubator at 25°C. in darkness for thirteen days and then exposed to light (from 100-watt electric bulb) for 20, 40 and 60 minutes, and placed back at 25°C. in darkness for five days. The results are shown in Table V. It was noticed that similar exposures to daylight did not give satisfactory results.



(For explanation see p. 529.)

TABLE V.

Effect of light on zonation in C. dolichi *E. and E.*

Agar medium	Plates thin or thick	Position of the plates	Ordinary day light condition	Continuous light	Continuous darkness	Darkness with electric light exposures of—		
						20 min.	40 min.	1 hour
Coons'	Thick	Upright	X					
		Inverted	XX	—	—	—	X	XX
	Thin	Upright	XX					
		Inverted	XXX	—	—	—	XX	XXX
Oatmeal	Thick	Upright	X					
		Inverted	XX	—	—	—	—	X
	Thin	Upright	XX					
		Inverted	XXX	—	—	X	X	XX
Richards' solution *N*/5	Thick	Upright	X					
		Inverted	XX	—	—	—	—	X
	Thin	Upright	XX					
		Inverted	XXX	—	—	—	X	XX

— = No zonation. X = Slight. XX = Moderate. XXX = Good.

(*ii*) *Fluctuating temperature.*—Three inoculated plates each of thick and thin media were placed at constant temperatures of 18°C. and 31°C. while another set was first kept for six days at 18°C., then for four days at 31°C., and again for six days at 18°C. (Plate XXXIV, fig. 2). A third set of plates was first kept for six days at 31°C. then for four days at 18°C., and again for six days at 31°C. This experiment was repeated with three different media and the results are given in Table VI. Fluctuating temparatures help in the formation of zones. Prominent zones are formed by distinctly low and high fluctuating temperatures while no zones are formed at constant temperatures.

TABLE VI.

Temperature in relation to zonation in C. dolichi *E. and E.*

Agar medium	Amount of media	18°C. constant	31°C. constant	18°C.-31°C. alternating	31°C.-18°C. alternating
Coons'	Thick	—	—	X	X
	Thin	—	—	XXX	XXX
Richards' solution *N*/5	Thick	—	—	X	X
	Thin	—	—	XX	XX
Oatmeal	Thick	—	—	X	X
	Thin	—	—	XX	XX

— = No zonation; X = Slight; XX = Moderate; XXX = Good.

(*iii*) *Effect of dilution of rich media on zonation.*—Zone formation on Richards' solution agar (normal strength) was compared with those formed on Richards' solution agar *N*/2-*N*/100. The data given in Table VII show that by diluting rich media zone formation becomes more prominent up to a certain dilution beyond which no zone formation takes place. Mitra [1931, 2] also found absence of zones on normal Richards' solution agar except on one strain of *Helminthosporium*. Abundant aerial mycelium does not permit the formation of zones on rich media because submerged mycelium is not affected. In the case of *C. dolichi* E. and E. best zones are formed at *N*/5 while there is no zonation at *N*/100.

TABLE VII.

Effect of different concentrations of Richards' solution agar on zonation of C. dolichi *E. and E.*

Concentrations	Thick plates	Thin plates
N	X	XX
N/2	XX	XXX
N/5	XXX	XXXX
N/10	X	XX
N/20	X	XX
N/50	—	X
N/100	—	—

— =No zonation; X=Slight; XX=Moderate; XXX=Good; XXXX=Very good.

The amount of media does not appear to be the causative factor in the formation of zones, since no zones are formed in thin plates kept at constant temperatures whether in light or darkness. Zonation becomes more distinct at higher humidities but humidity alone has no effect on zone formation (Plate XXXIV, fig. 3).

(*f*) *Concentration.*

C. dolichi E. and E. was cultured on Coons' solution 10*N*, 5*N*, 2*N*, *N*, *N*/2, *N*/5, *N*/10, *N*/20, *N*/50, and *N*/100 in flasks of uniform capacity containing 100 c.c. of the medium. Inoculated flasks were kept at 27·5°C. and the mycelium was filtered after 27 days and the dry weight of the fungus determined. The experiment was run in triplicate and the data obtained are shown in Table VIII. The greatest growth was at 10*N* and least at *N*/20, N/50 and *N*/100; the dry weight of the mycelium thus increased in direct proportion to the increase in concentration of the medium. At higher concentrations a strongly wrinkled weft of mycelium is developed a considerable part of which stands exposed above the liquid and is coloured rose neyron. From 10*N*-*N*, no trace of submerged colonies is found. With a decrease in concentration there is reduction in the amount and texture of the aerial mycelium.

TABLE VIII.

Average dry weight of the mycelium (in grm.) of C. dolichi *E. and E. at various concentrations of Coons' synthetic solution after twenty-seven days at 27·5°C.*

Concentrations	10*N*	5*N*	2*N*	*N*	*N*/2	*N*/5	*N*/10	*N*/20	*N*/50	*N*/100
Average dry weight of the mycelium (in grm.)	0·6896	0·3914	0·1372	0·0789	0·0270	0·0045	0·0018	0·0011	0·0008	0·0004

The results obtained agree with those of Moore [1924] and Brown [1925].

On the contrary it was found that with a solid medium such as Richards' solution agar best growth occurred at normal concentration. The growth rate decreases either with the increase or decrease of concentration. Table IX gives the average of three readings after 9, 12 and 15 days.

TABLE IX.

Average linear growth rate in mm. of C. dolichi *E. and E. at various concentrations of Richards' solution agar at 27·5°C.*

Days	10*N*	5*N*	2*N*	*N*	*N*/5	*N*/10	*N*/20	*N*/50	*N*/100
9	9·3	21·6	41	47	41	39	37·6	37	35
12	12·3	38·3	60	63	52	50	49	48	46
15	14	50	71·3	73	62	60	58	57	56

(*g*) *Importance of different constituents of a synthetic medium.*

In order to test the importance of various salts of a nutrient medium with regard to the growth of *C. dolichi*, the various constituents of Doxs' solution were tested by eliminating each salt one by one. The data thus obtained are given in Table X.

TABLE X.

Average dry weight of the mycelium of C. dolichi *grown on Doxs' solution and those lacking in one of its constituents at 27·5°C. after 65 days.*

Media	Dry weight of the mycelium in grm.
Normal (*N*)	0·125
N KH_2PO_4	0·0005
N Cane sugar	0 0017
N KNO_3	0·089
N $MgSO_4$	0·096
N KCl	0·102
N $FeSO_4$	0·122

It will be seen from Table X that the importance of KH_2PO_4 is greatest, while cane sugar comes next in order. $MgSO_4$, KCl and $FeSO_4$ are of less importance.

(*h*) *Effect of different sugars.*

The effect of different sugars on the dry weight of the mycelium was studied in a 5 per cent. solution of various sugars. The sugars used were maltose, glucose, sucrose, lactose and lævulose. Triplicate flasks, each containing 100 c.c. of the various sugar solutions used for each series, were filtered after fifty days, and the results are shown in Table XI. Best growth takes place on maltose solution and least on lactose solution.

TABLE XI.

Dry weight of the mycelium of C. dolichi *on different sugar solutions at 27·5°C. after fifty days.*

5 per cent. solution	Average dry weight of the mycelium (grm.)	pH	pH control after 50 days	pH inoculated after 50 days
Maltose	0·0872	5·3	5·3	5·0
Glucose	0·0642	5·1	5·0	4·0
Sucrose	0·0415	6·7	5·7	5·2
Lævulose	0·0095	4·5	4·5	4·3
Lactose	0·0062	5·1	5·1	4·2

(*i*) *Temperature.*

The temperature of different regions, aside from other climatic conditions, may greatly affect the occurrence of the parasites. Fawcett [1917, 1] has shown the correlation existing between the cardinal temperatures of certain fungi and seasonal occurrence. The importance of low temperatures in the preservation of fruits is well known, and high temperatures are employed not only to regulate ripening and sweetening processes but also to effect the death of the invading parasites without lowering the vitality of the host. Stevens [1917] has shown that the rate of increase of chestnut blight canker is closely related to temperature. According to Tisdale [1917] the temperature at which the host is most injured by *Fusarium* wilt of flax corresponds to that favouring the maximum growth of the parasite in cultures. Humphrey [1914] came to the conclusion that the temperature differences in various localities in the State of Washington largely determined the differences in distribution and severity of tomato wilt induced

by *Fusarium orysporium*. Fawcett [1917] referred to the limited geographical distribution of melonose due to *Phomopsis citri* and suggested that temperature may be among the important factors limiting its distribution.

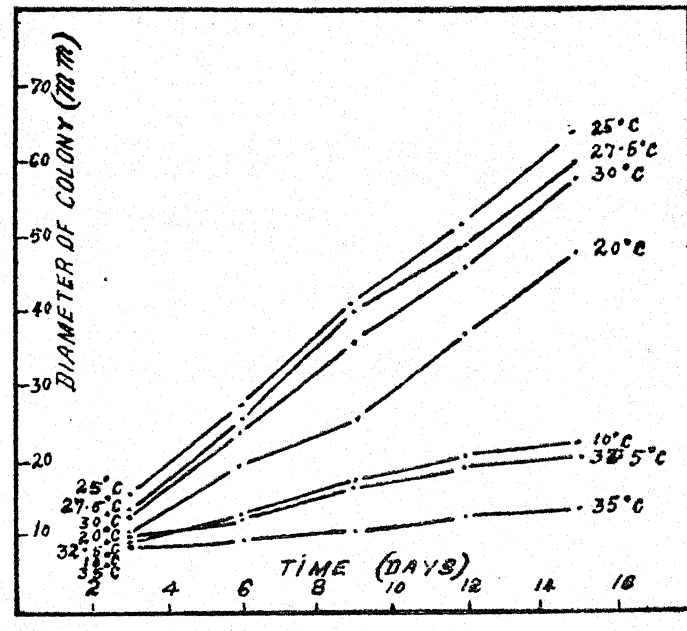


Fig. 2.—Temperature relationship of *C. dolichi* on Coons' agar.

In the study of temperature relationship of a given organism it is necessary to mention other conditions that are supposed to be effective, *i.e.*, the nature of the medium, the length of time, the uniformity in the amount of the medium.

A study of the temperature relationship of *C. dolichi* was made on six different media, *viz.*, Coons' agar, oatmeal agar, *Dolichos Lablab* leaf decoction agar, Hopkins' agar, Richards' solution agar and potato synthetic agar. Measurements of the colonies were taken after three days' interval. Triplicate plates of uniform size each containing about 50 c.c. of the medium were placed in each of the following temperatures—5·5°C., 10°C., 20°C., 25°C., 27·5°C., 30°C., 32·5°C., 35°C., and 37·5°C. No growth took place at 5·5°C. and 37·5°C. except on Richards' solution agar at 37·5° C. The optimum temperature for growth was 25°C. in all cases

except Richards' solution agar for which it was 27·5°C. The shifting of the optimum as well as the maximum in media having a high carbon/nitrogen ratio is not unknown. Weimer and Harter [1923] in their study of temperature relationship of eleven species of *Rhizopus* observed that the presence of 20 per cent. dextrose in Irish potato agar changed the cardinal temperatures of a strain of *nigricans* studied from 1°C. to 2°C. Similar results were obtained by Thiele and Rudolf [1896]; the addition of dextrose to glycerine and formic acid changed the maximum temperature for growth of one species of both *Penicillium* and *Aspergillus*.

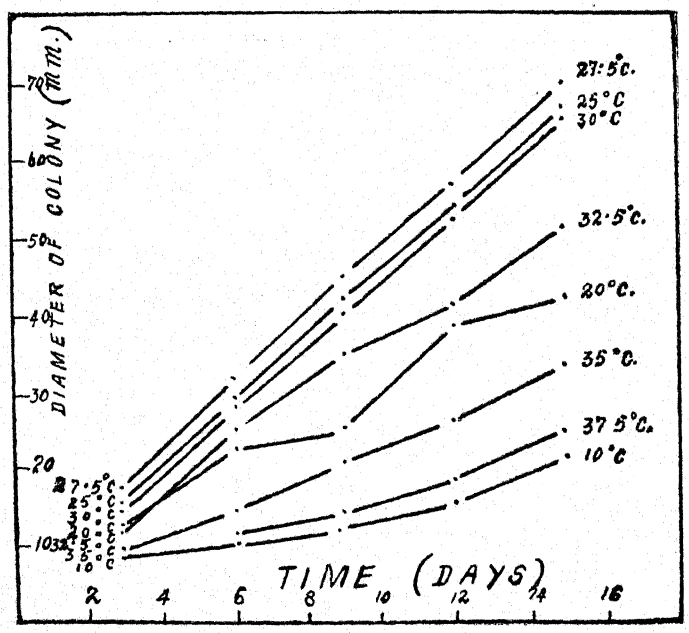


Fig. 3.—Temperature relationship of *C. dolichi* on Richards' solution agar.

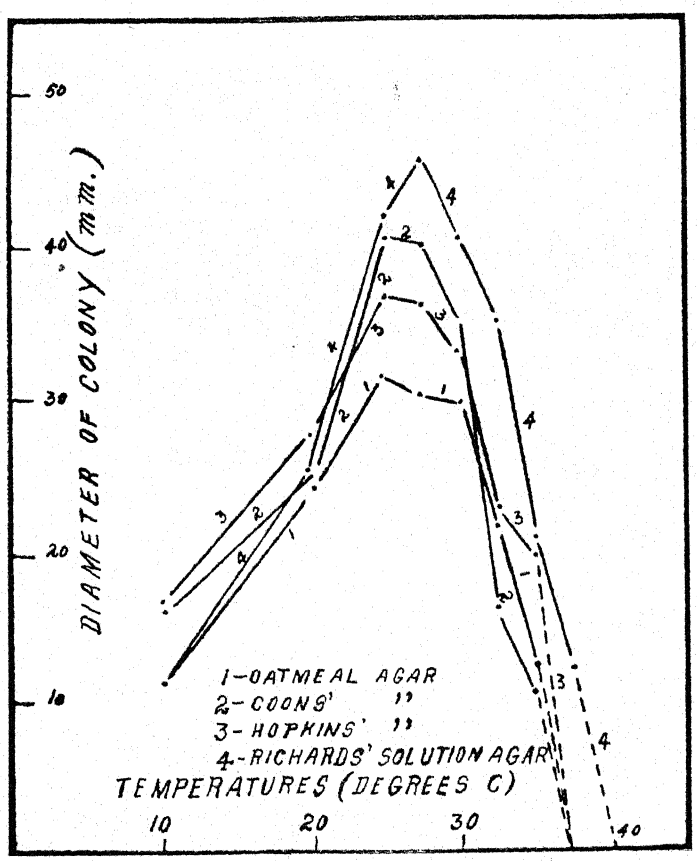


Fig. 4.—Nine days' growth of *C. dolichi* at various temperatures on four different media.

It was noticed that at low temperatures the linear rate of growth goes on increasing uniformly but at higher temperatures above the optimum this uniform growth is checked and the cultures show a tendency of staling.

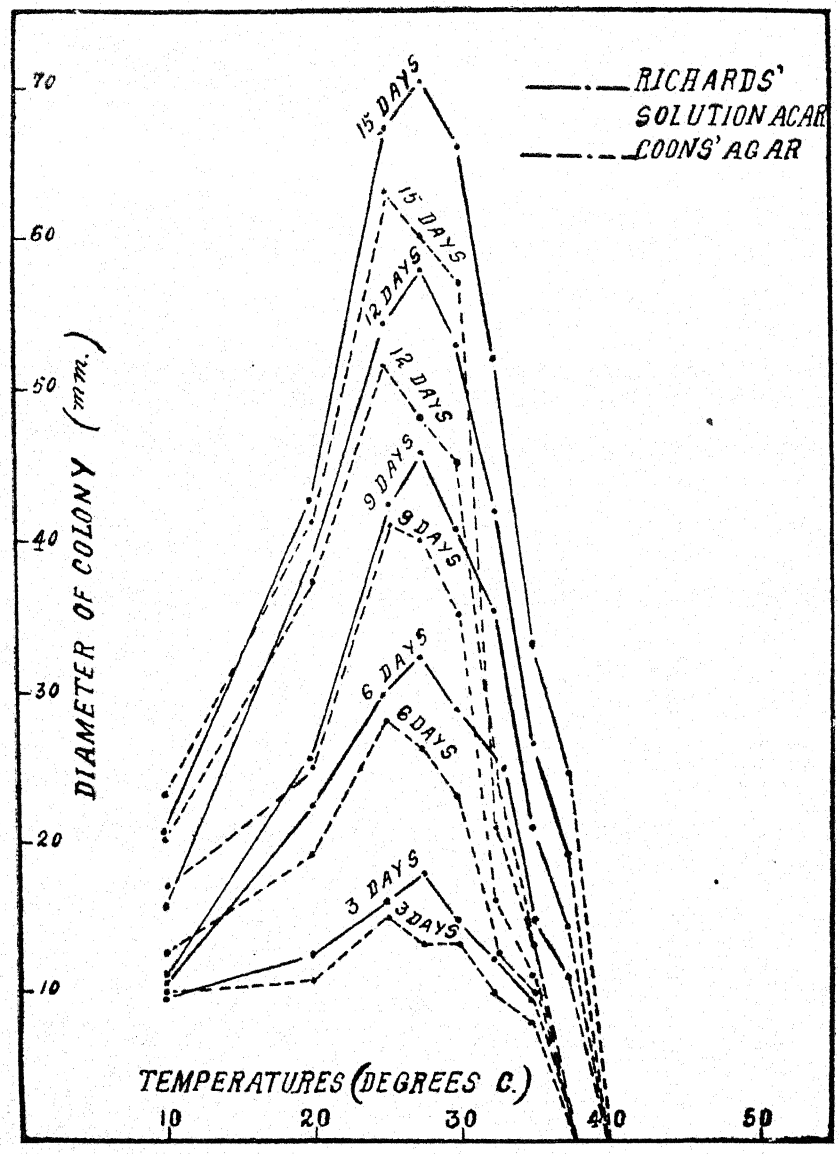


Fig. 5.—Growth of *C. dolichi* at various temperatures on Richards' solution agar and Coons' agar.

Thus at higher temperatures growth is very poor. Figs. 2 and 3 show the growth rate at various temperatures on Coons' agar and Richards' solution agar, and figs. 4 and 5 show the relation between the growth rate on four different media and the time from the beginning at a given constant temperature.

It will be seen from these graphs that the fungus grows well between 20°C. and 30°C. and that temperatures above or below these limits are detrimental to the best growth of the fungus. At higher temperatures an abnormal type of growth takes place and the colony becomes pale and forms a lot of felty ærial mycelium.

(B) MICROSCOPIC CHARACTERS.

(*a*) *Character of the mycelium.*

The mycelium when young is septate at long intervals and is sub-hyaline; with age it becomes light brown to greenish brown and septa appear at short intervals; later on chlanydospores were formed on some media such as oatmeal agar, Coons' agar, etc. (Plate XXXIII, figs. 15-18). Sclerotial bodies are formed in very old cultures and are numerous on *Dolichos* stem and wheat straw.

(*b*) *Size and degree of septation of spores.*

C. dolichi does not sporulate on oatmeal agar, Coons' agar, Hopkins' agar, *Dolichos* leaf decoction agar and prune juice agar, but does so on Richards' solution agar, *Dolichos* stem and on wheat straw. The spores formed on wheat straw and *Dolichos* stem are very long and thin but not profuse as on Richards' solution agar. Scratching of the surface of the culture with a sterile needle often stimulates the formation of spores. Sporulation is better in complete darkness than in alternate light and darkness.

(*i*) *Effect of temperature.*—Temperature variation is the most important factor in affecting the size and septation of spores. The shape of the spores is, however, not much affected except that the scars on the proximal end are not so marked as in spores formed in nature. Spore formation is very poor at temperatures below 20°C. and above 30°C., and at 27·5°C. (the temperature of optimum growth) the spores formed are largest in size and septation (Plate XXXIII, figs. 25-32). At 10°C., 32·5°C. and 35°C. the spores formed are abnormally small in size and with few septa (Plate XXXIII, figs. 19-24). The spore size and septation at different temperatures are given in the Table XII.

TABLE XII.

Variation in size and septation of spores of C. dolichi *on Richards' solution agar at various temperatures.*

Temperature	Range of length (μ)	Average length (μ)	Range of width (μ)	Average width (μ)	Range of septation	Average septation	Septation mode
10°C.	6·8-51·0	20± ·7	1·7-2·5	1·9	0-3	1·3	1
20°C.	6·8-91·8	36±1·3	2·5-5·1	4·1	0-7	2·4	2
25°C.	6·8-187	46±2·6	1·7-5·1	3·6	0-12	3·8	3
27·5°C.	20·4-200·6	68·5±2·4	3·4-6·8	4·1	0-14	5·4	4
30°C.	7·2-102	46±1·7	1·7-5·2	2·5	0-8	3·0	3
32·5°C.	3·4-74·8	14± ·7	1·7-3·4	1·8	0-6	1·0	1

(*iii*) *Effect of different media.*—The size and septation of spores depend on the nature of the medium on which the fungus is cultivated. Spore measurements were made from fifteen days old cultures grown at 20°C. on Richards' solution agar, *Dolichos* stem and wheat straw, and are shown in Table XIII.

TABLE XIII.

Variation in size and septation of spores of C. dolichi *on different media at 20°C.*

Media	Range of length (μ)	Average length (μ)	Range of width (μ)	Average width (μ)	Range of septation	Average septation	Septation mode
Richards' solution agar	6·8-91·8	36±1·3	2·5-5·1	4·1	0-7	2·4	2
Dolichos stem .	22·8-208	103±·7	3·2-4·8	4·5	0-17	8·5	7
Wheat straw .	32·0-272	85±1·0	3·2-4·8	4·0	0-15	10	9

It will be seen from Table XIII that the septation of spores under identical conditions is greatest on wheat straw and least on Richards' solution agar. The greatest average length of spores is on *Dolichos* stem and least on Richards' solution agar.

(*iii*) *Effect of different relative humidities.*—Welles [1925], Lehman [1928], Klotz [1923], Sundararaman and Ramakrishnan [1928], and Ramakrishnan [1931] found on different species of *Cercospora* that remarkable increase in size and septation of conidiophores and conidia take place at high humidities. An experiment was carried out by incubating infected leaves at relative humidities of 47, 70·4 and 100, and after forty-eight hours a count of hundred conidia was made.

TABLE XIV.

Effect of different relative humidities on size and septation of spores.

Relative humidity	Range of length (μ)	Average length (μ)	Range of width (μ)	Average width (μ)	Range of septation	Average septation	Septation mode
47	17-163	79±2·4	3·4-6·8	4·8	0-11	6·0	4
70·4	20·4-268	91±1·2	3·4-5·1	4·5	0-18	7·5	5
100	17-306	114±1·0	3·4-5·1	4·5	0-21	8·1	7

It will be seen from the above table that the average length of conidia increased and the average breadth decreased with the rise in relative humidity. Hence in determining measurements of spores of any species of the genus *Cercospora* relative humidity and temperature should be taken into consideration.

(*c*) *Secondary spores.*

The formation of secondary conidia from primary conidia often takes place both in nature as well as in culture. In some cases the first-formed conidium produced a second one at its tip, while in others lateral conidia are formed from the sides of the basal cells of the larger conidia (Plate XXXIII, fig. 33). The secondary conidia thus formed are small and usually unseptate, rarely one septate. Such secondary conidia formation is a common characteristic of most of the allied fungi such as *Helminthosporium*, etc.

(*d*) *Spore germination.*

The conidia readily germinate in tap water within two hours. Germ tubes are given off from both ends of spores as well as from sides near the septa. At times two germ tubes originate from a single cell of a conidium. Frequently conidia in cultures germinate *in situ*. Conidiophores and even bits of them also germinate readily in tap water. Spores from specimens two months old do not germinate ; the viability of the conidia thus lasts for a very short time only. Lehman [1928] however, reports that the conidia of *Cercospora diazumaru* germinate after 69 days but according to Klotz [1923] those of *Capii* Fres., after 170 days. Spore germination is affected by the following factors :—(1) Temperature (ii) carbon dioxide (iii) light and darkness (iv) hydrogen-ion concentration.

(*i*) *Effect of various temperatures on germination.*—Spores were taken from a twenty-five days old culture on *Dolichos* stem, and spores suspension made in sterilized distilled water. A drop of the spore suspension was placed in the centre of a cover slip and inverted over Van Tiegham rings containing a few drops of distilled water. These were then exposed for five hours at 5·5°C., 10°C., 18°C., 25°C., 31°C., 35°C. and 41°C. temperatures. Percentage germination of spores and the average lengths of the germ tubes for each temperature were determined from a count of fifty for each.

TABLE XV.

Spore germination of C. dolichi *at various temperatures.*

Temperature	Percentage germination	Average length of germ tubes (μ)
5·5°C.	0	0
10°C.	34	12·6
18°C.	74	20·7
25°C.	80	40·0
31°C.	58	32·0
35°C.	36	15·6
41°C.	0	0

It therefore follows that there is an increase in the percentage of germination as well as in the average length of the germ tubes, from 10°C.-25°C. At 25°C. the percentage of germination as well as the average length of the germ tubes is greatest; it is thus the optimum temperature for spore germination. The minimum temperature for spore germination lies between 5·5°C. and 10°C., while the maximum lies between 35°C. and 41°C. Spores are killed when exposed for five hours at 41°C. Hence the thermal death point lies in the neighbourhood of 41°C.

(*ii*) *Effect of 100 per cent. carbon dioxide on germination.*—Carbon dioxide is known to have a retarding effect on the growth and the germination of conidia of fungi. An attempt was made to see the effect of 100 per cent. carbon dioxide on germination. Spores in hanging drops were mounted in ward tubes and a stream of 100 per cent. carbon dioxide was passed for two to three minutes sufficient to displace completely inside air and then the two ends were closed tightly with stopcocks. The cells being of uniform size, there was an equal amount of carbon dioxide in all tubes. Controls were kept with ordinary air inside them. These tubes were all kept at 25°C. The percentage germination as well as the average length of the germ tubes were measured after five hours.

TABLE XVI.

Effect of 100 per cent. carbon dioxide on spore germination of C. dolichi *at 25° C.*

Spores exposed for five hours in	Percentage of germination	Average length of the germ tubes
Ordinary air	88	22·0μ
100per cent. carbon dioxide	30	9·8μ

An atmosphere of 100 per cent. carbon dioxide had a retarding effect on the percentage of germination as well as the average length of the germ tubes. There was also no branching of the germ tubes in spores exposed to 100 per cent. carbon dioxide.

(*iii*) *Effect of continuous light and continuous darkness on germination.*—An experiment was carried out to see the effect of continuous light and continuous darkness on the germination of spores but no conclusive results were obtained.

(*iv*) *Effect of hydrogen-ion concentration on germination.*—Spore germination is remarkably affected by hydrogen-ion concentration of the solution. Spore suspensions were made in solutions of known pH values and a drop mounted on a cover

slip and inverted over van Tiegham rings containing a few drops of solution of the same pH value as of the hanging drop. Adjusted modified Richards' solution of Karrer and Webb [1920] was used and the solutions of the following pH values were prepared :—2·1, 2·5, 2·7, 2·8, 2·9, 4·7, 4·9, 5·1, 5·9, 6·3, 6·9, 7·3, 8·0, and 9·1. All the slides were incubated at 25°C. for five hours. Then a drop of one per cent. mercuric chloride was put in each hanging drop in order to fix the spores germinated and ungerminated. The percentage of germination and the average length of the germ tubes were determined for each pH value. The figures given below are average of fifty counts for each.

TABLE XVII.

Effect of different H- and OH-ion concentrations on germinations of C. dolichi *at 25° C.*

pH values	2·1-2·8	2·9	3·3	4·7	4·9	5·1	5·9	6·3	6·9	7·3	8·0	9·1
Percentage of germination	..	32	48	56	68	96	98	80	22	12	8	0
Average length of the germ tubes (μ)	..	11·4	12·0	12·7	15·2	24·7	27·3	17·0	10·5	2·4	2·0	0

Evidently the spores can tolerate a wide range of hydrogen-ion concentration from 2·9-8·0. The germination begins abruptly at pH 2·9 and the optimum is at pH 5·9. The minimum hydrogen-ion concentration for germination lies between pH 2·8 and 2·9, while the maximum lies between pH 8·0 and 9·1. Germination is a process which is strikingly supported by a relatively high hydrogen-ion concentration as shown by Webb [1919-1921]; hence hydroxyl-ions are more toxic than hydrogen-ions as found by Clark [1899] who also concluded that with an increase in the length of interval of incubation, the reaction of germination to hydrogen-ion concentration remains practically the same, the curves for germination for any organism being practically identical whether incubated at 22° C. or 27° C. or 30°C.

IV. Hydrogen-ion concentration.

In order to determine the hydrogen-ion concentration relationship to the growth of *C. dolichi*, Richards' solution agar as modified by Karrer and Webb [1920] was used and his method was followed. 30 c. c. of the solution together with the required amount of *N*/5 acid and *N*/5 alkali and distilled water to make 50 c.c. was put in each flask and hydrogen-ion concentration was determined according to the

colorimetric method of Clark and Lubs [1917] and the range taken was pH 2·1-8·5. Flasks were incubated after inoculation for sixty-two days at 27·5°C. and the dry weight of the mycelium of the fungus determined. The hydrogen-ion concentrations of the controls and filtrate were also determined to see whether changes were brought about as a result of the metabolic activities of the fungus. The data obtained are given in the table below and represent the average dry weight of the mycelium from three flasks in each case.

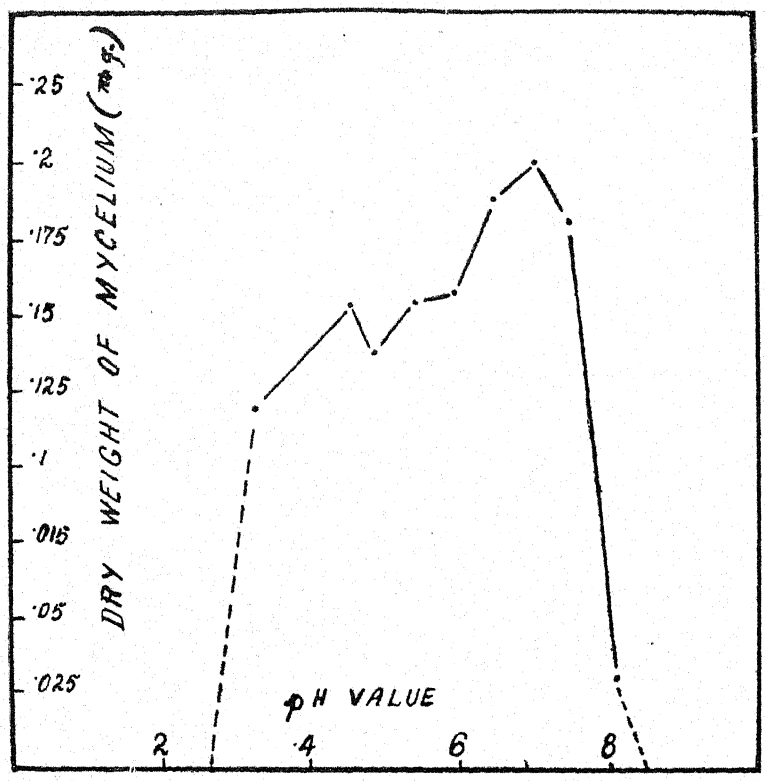


Fig. 6.—Growth of *C. delichi* on modified Richards' solution agar on different H-ion concentration after 62 days at 27·5°C.

TABLE XVIII.

Growth of C. dolichi *and the changes in reaction induced by growth in modified Richards' solution at different pH values at 27·5°C. in darkness after 62 days.*

H- AND OH-ION CONCENTRATIONS			Average dry weight of the mycelium (grm.)	Remarks
Initial	Control after 62 days	Inoculated after 62 days		
2·1	2·1	2·1	No growth	No growth
2·5	2·5	2·5	Do.	Do.
2·7	2·7	2·7	Do.	Do.
3·3	3·3	2·3	0·1189	Growth moderate. Numerous small floating colonies. Submerged colonies few.
4·6	4·1	2·3	0·1533	Growth more than pH 3·3 and 4·9.
4·9	4·5	2·3	0·1339	Growth poor, less than pH 4·6. Few floating colonies. Submerged colonies thin and filmy.
5·5	4·7	2·3	0·1535	Big floating colonies.
6·0	5·1	2·3	0·1643	Numerous floating colonies. Submerged colonies thin and filmy.
6·5	5·5	2·3	0·1890	Same as in pH 6·0.
7·1	6·3	2·3	0·2004	Numerous small floating colonies. Submerged colonies few and filmy.
7·5	6·1	2·9	0·1816	Only one big floating colony. Numerous big submerged colonies.
8·1	6·1	6·5	0·0281	Growth very poor, in traces only. No floating colonies.
8·5	6·8	6·8	No growth	No growth.

There is no growth at higher hydrogen-ion concentrations than pH 3·3 but growth abruptly starts from pH 3·3 and goes on up to pH 8·1 (Table XVIII and Fig. 6). Two maxima were obtained one on acid side at pH 4·6 and the other on the alkali side near neutrality at pH 7·1. Double maxima have been reported for a number of fungi and are by no means uncommon. pH 2·1, 2·5, 2·7 and 8·5 should be regarded as toxic hydrogen-ion and hydroxyl-ion concentrations for the growth of this fungus, while pH 8·1 may be regarded as inhibitory hydroxyl-ion, because when pH 8·5 was reduced to pH 6·5, a slight growth took place though in traces only. The fungus during its growth on the modified Richards' solution produced marked changes in the reaction of the medium. It will be seen from Table XVIII that there has been very little change in the pH value of the controls on the acid sides while on the alkali side there is a remarkable shift in pH values. The pH values of 3·3, 4·6, 4·9, 5·5, 6·0, 6·5 and 7·1 of the inoculated flasks shifted to pH 2·3 but pH 7·5, however, shifted to pH 2·9 while pH 8·1 and 8·5 to pH 6·5 and 6·8 respectively. It appears that the fungus requires for its best growth an acid medium or a slightly alkaline one. Too high hydrogen-ion and too low hydroxyl-ion concentrations are harmful to the best growth of the fungus. Since the fungus can grow

up to as high a pH value as 8·1, it is clear that it is by no means impossible for the dormant mycelium in the leaves to live in the Pusa soil of which the pH value is about 8·2. It has been shown by numerous workers that the influence of hydrogen-ion concentration on the vegetative growth of the fungus in pure cultures is variable and to a large extent dependent on the chemical composition and physical nature of the medium. There is every possibility that pH relations of the fungi in the soil may be slightly different from those obtained by using artificial medium but considering that the limiting hydrogen-ion concentration is the same as obtained in artificial media,it is quite possible for the dormant mycelium to germinate with the return of the favourable conditions to form new crop of conidia for propagation. If by some means, say by adding lime, etc., as recommended by numerous workers, the soil could be made alkaline, it is quite possible to kill the dormant mycelium in the trash lying in the soil.

A study of the growth of the fungus on modified Richards' solution with a pH value of 4·5 was carried out and records were made at an interval of 10 days in the shift in pH values of the inoculated and control flasks. The data given in Table XIX show that there is no change in the pH value of the controls after ten days but in twenty days it dropped to 4·3 and in fifty days to pH 4·1. Thus there was an increase in acidity in control flasks from pH 4·5-4·1 in fifty days. The acidity of inoculated flasks, on the other hand, changed from pH 4·5 to pH 2·3 in thirty days. There is greatest increase in the dry weight of the mycelium between twenty and thirty days and an appreciable increase in acidity after 20 days.

TABLE XIX.

Growth of C. dolichi, *and changes in reaction when grown on modified Richards' solution of pH 4·5, after intervals of ten days up to fifty days at 30°C.*

Days	pH of control	Average pH of filtrate from inoculated flasks	Average dry weight of the mycelium (grm.)
10	4·5	3·0	0·1217
20	4·3	2·5	0·1383
30	4·3	2·3	0·2722
40	4·2	2·3	0·2731
50	4·1	2·2	0·2775

After thirty days there is little increase in the dry weight of the mycelium and very little decrease in the pH value of the solution.

V. Infectivity.

Infection takes place readily both with mycelium and spores. In the case of mycelium as inocula, infection takes place within seventy-two hours and with

spores infection spots begin to appear after five to seven days. The infection readily takes place from the under surface of leaves, and mature leaves are more susceptible to infection than immature leaves. Inoculated leaves were incubated and kept under various humidities, and it was observed that no infection takes place below 79 per cent. humidity. The details of inoculation experiments are given in Table XX.

TABLE XX.

Summary of the results of inoculation experiments on Dolichos Lablab *by* C. dolichi *E. and E.*

No. of experiments	Part of host inoculated	No. of inoculation	No. of infection	Percentage of infection
1	Leaves—			
	Upper surface . .	14	1	7·1
	Under „ . .	30	8	26·6
2	Leaves—			
	Upper surface . .	15	4	26·6
	Under „ . .	56	34	63·5
3	Leaves—			
	Upper surface . .	16	3	18·7
	Under „ . .	32	17	53·0
4	Leaves—			
	Upper surface . .	28	7	25·0
	Under „ . .	70	39	55·7
	Stems . . .	5	4	80·0
5	Leaves—			
	Upper surface . .	6	1	16·6
	Under „ . .	30	10	33·3
6	Leaves—			
	Under surface . .	73	28	38·3
	Petioles . . .	5	2	40
	Stems . . .	5	3	60
7	Leaves—			
	Under surface . .	80	46	57·6
	Stems . . .	40	24	60
8	Petioles . . .	80	32	40
	Stem . . .	70	25	35·5

C. dolichi is able to infect all parts of *Dolichos Lablab*, pods and seedlings also readily take infection from spores.

Cross inoculation.—The parsitism of *C. dolichi* is not restricted to *Dolichos Lablab*; it readily infects a number of other cultivated Indian pulses. Details of various cross-inoculation experiments carried out are given in Table XXI.

TABLE XXI.

Cross-inoculation experiments with C. dolichi.

No. of experiment	Date	Hosts	Part of hosts inoculated	No. of inoculation	No. of infection	Percentage infection	Remarks
1	July 1931—4th	*Glycine hispida* (*soybean*)	Leaves (under surface)	26	11	54	Plants kept under bell jar
2	6th	*Ditto* .	,,	24	16		,,
3	17th	*Phaseolus aconitifolius* (*moth*)	,,	50	21	42	,,
4	18th	*Vigna catjang* (*cow pea*)	,,	50	30	60	Cut stem under bell jar
5	24th	*Cajanus indicus* (*rahar*)	,,	50	15	30	,,
6	26th	*Phaseolus mungo* (*urid*)	,,	50	20	40	,,
7	28th	*Phaseolus radiatus* (*mung*)	,,	50	12	24	,,
8	30th	*Ditto* .	,,	50	10	20	,,

VI.—Saltation.

The formation of sectors, many of which proved later on to be saltants of differently coloured mycelium in the colonies grown on nutrient media, is of very common occurrence. This sectoring takes place on a large number of media of varying constituents, especially on Coons' agar and Richards' solution agar. These saltants also arise from inocula taken at random from different portions of a colony. Different factors found to be responsible for the formation of sectors were :—(*i*) the shallowness of the medium, (*ii*) the richness of the medium, (*iii*) temparature, (*iv*) the concentration of the medium and (*v*) the position from which the inocula was taken.

(*i*) *Shallowness of the medium.*

Sector formation takes place more on thin plates or on thin side of slant plates than on thick plates or on the thicker side of slant plates. Similar results

were obtained by Christensen [1929] on *Helminthosporium sativum*, Mitra [1931, 2] on *Helminthosporium* spp. and Brown [1926] on *Fusarium*. In shallow medium the differences between the parent and the saltant are magnified but it cannot be claimed that shallow medium is the cause of saltation. Saltants are present in thick plates but in hidden form.

(ii) Richness of the medium.

Sector formation takes place much more on rich media such as Coons' agar, Richards' solution agar than on poor media such as Browns' synthetic agar, Beyrincks' agar, etc.

(iii) Concentration of the medium.

Thick, thin and slant plates of Richards' solution agar and Coons' agar of *N*, *N*/5, *N*/10, *N*/20, *N*/50 and *N*/100 concentrations were inoculated and kept at constant temperatures of 32·5°C. and 27·5°C. respectively. Triplicate plates for each series were kept. These were examined after forty days. The number of sectors formed in each type of plates at various concentrations were counted and the data are given in the Table XXII.

TABLE XXII.

Number of sectors formed by C. dolichi *at various concentrations of Richards' solution agar and Coons' agar.*

Medium	Amount of media	2 *N*	*N*	*N*/5	*N*/10	*N*/20	*N*/50	*N*/100
Richards' solution agar 32·5°C.	Thin	—	10	3	1	1	0	0
	Thick	—	0	1	0	0	0	0
	Slant Thin	—	—	—	0	0	0	0
	Slant Thick	—	—	—	0	0	0	0
Coons' agar 27·5°C.	Thin	20	15	—	—	—	0	0
	Thick	5	2	—	—	—	0	0
	Slant Thin	8	9	—	—	—	0	0
	Slant Thick	3	1	—	—	—	0	0

It will be seen from Table XXII that the formation of sectors is greater at higher concentrations than at lower and there is no formation of sectors at *N*/50 and *N*/100 concentrations; the number of sectors formed on thinly poured plates is greater than on thickly poured plates and more on the thinner side than on the thicker side

of slant plates. The concentration and richness of medium are thus responsible for the formation of saltants.

(*iv*) *Temperature.*

Thin, thick and slant plates of Coons' agar and Richards' solution agar were kept at 10°C., 18°C., 25°C., 27·5°C., 30°C., 32·5°C., 35°C. and 37·5°C. Triplicate plates were used for each series. These were examined after one month. On Coons' agar no sectors were formed at 10°C., 35°C. and 37·5°C., but most sector formation took place between 25°C. and 27·5°C. On Richards' solution agar there were no sectors at 10°C., 18°C. and 37·5°C. but most sector formation took place between 27·5°C. and 32·5°C. and a few sectors formed at 35°C. Thus the optimum temperature for sector formation in the case of Coons' agar lies between 25°C. and 27·5°C. while in the case of Richards' solution agar between 27·5°C. and 32·5°C. At lower temperatures sectors are formed late and in lesser number because the activity of the fungus is less while at very low temperatures (say 10°C.) the fungal activity being almost arrested, the sectors do not appear at all.

(*v*) *Position from which inocula are taken.*

The next experiment related to the determination of the portion of a fungal colony containing the largest number of saltants. Thick plates of Richards' solution agar of *N*, *N*/50 and *N*/100 were kept for forty days at 32·5°C. and inocula concentrations from these plates, fourteen from the middle and six from the margin, were taken and planted in the centre of plates of Coons' agar and incubated at 32·5°C. and examined after one month. The data obtained are given in the table below.

TABLE XXIII.

Number of sector formation from inocula taken from different regions of a colony on Coons' agar at 32·5°C.

Concentrations of Richards' solution agar	Middle	Margin
N	13	0
N/50	6	2
N/100	4	2

The number of sectors obtained from inocula taken from the middle region of the colony was larger than the number of sectors obtained from inocula taken from the marginal region. The formation of sectors from inocula taken from

C. dolichi grown on Richards' solution agar of *N*/50 and *N*/100 concentrations was comparatively less.

Two saltants D_1 and D_2 which differed markedly from the parent D were compared with the parent as to (*i*) the nature of growth and character of aerial mycelium and submerged mycelium, (*ii*) the rate of linear growth, (*iii*) zonation, (*iv*) sporulation, (*v*) sclerotial formation, and (*vi*) pathogenecity. The data are given in Table XXIV below.

Saltant D_1 appeared as a definite sector on a thin plate of Richards' solution kept at 31°C. (Plate XXXIV, figs. 1 and 6.)

Saltant D_2 appeared as a definite sector on a thin plate of Coons' agar at 27·5°C., also on Richards' solution agar plate kept at 35°C. (Plate XXXIV, fig. 5.)

TABLE XXIV.

Comparison of the parent and saltants of C. dolichi.

——	Parent D	Saltant D_1	Saltant D_2
Nature and colour of aerial and submerged mycelium (Richards' solution agar).	(*i*) *Aerial mycelium*, woolly, light maize yellow; edge light cobalt blue.	(*i*) *Aerial mycelium*, low felty, pale rose pink.	(*i*) *Aerial mycelium*, felty, light hydrangia pink, edge light lilacy white.
	(*ii*) *Submerged mycelium*, light brown to greenish brown, greater in diameter, more tortuous with numerous small oil globules than D_1 and D_2.	(*ii*) *Submerged mycelium*, smaller in diameter, less tortuous and more hyaline with larger oil globules than in D.	(*ii*) *Submerged mycelium*, same as in D_1.
Colour of the substratum (Richards' solution agar).	Light severe blue; edge light greyish indigo.	Light salmon flesh .	Light ashy grey; edge light stone colour.
Sporulation	X	—	—
Zonation	XXX	XX	XX
Sclerotial formation	XX	—	X
Pathogenecity	XXX	XX	X

— =Absent; X=Slight; XX=Moderate; XXX=Good.

Both the saltants did not sporulate in any media, were less pathogenetic than the parent, and zonation as well as sclerotial formation were poor. The rate of

growth of saltant D_1 was greater than that of the parent, while that of D_2 was less than that of the parent (Fig. 7).

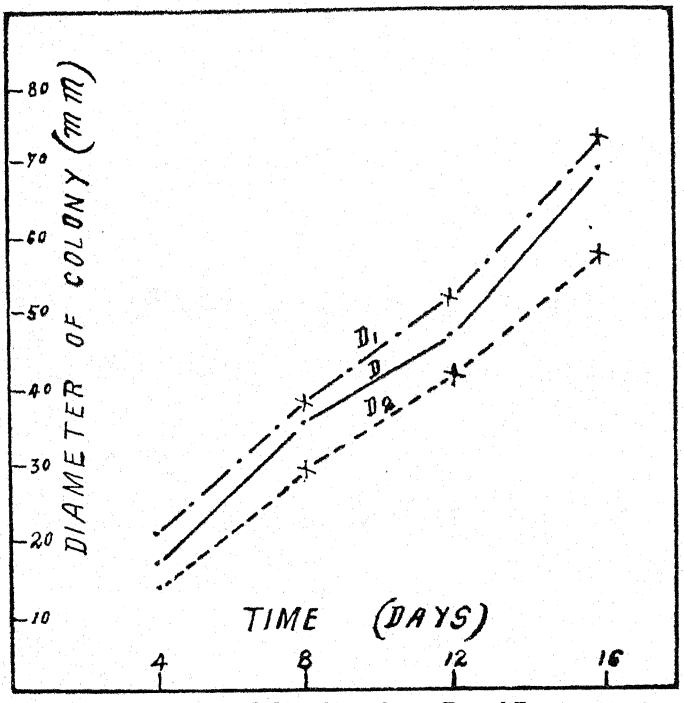


Fig. 7.—Linear rate of growth of *C. dolichi* and its saltants D_1 and D_2 on oatmeal agar at 27·5°C.

VII. Summary.

1. *Cercospora dolichi* E. and E. is parasitic on leaves, petioles, stems, pods and seeds of *Dolichos Lablab*. The morphology of the parasite is given in text.

2. A study of the fungus was made on a large number of artificial media. Its growth is better on thickly poured plates than on thinly poured plates; more in alternate light and darkness than in complete darkness or continuous light.

3. Alternate light and darkness and fluctuating low and high temperatures are the two important factors influencing zonation in artificial culture; other factors such as the amount of media and the concentration of media influence, but do not initiate zone formation.

4. It can tolerate a wide range of relative humidities from 47-100 per cent., the optimum humidity for growth being 78·7 per cent.

5. On Coons' solution 10 *N* N/100, the dry weight of the mycelium decreases with the decrease in concentration of the medium and on Richards' solution agar, the linear rate of growth decreases both by increasing and decreasing the normal concentration of the medium.

6. Potassium acid phosphate is the most important constituent of Doxs' solution influencing the growth of the fungus; cane sugar and potassium nitrate are next in importance in the order mentioned.

7. Growth was most abundant on 5 per cent. maltose solution and least on 5 per cent. lactose solution.

8. The optimum temperature for the growth of the fungus is 25°C. in all media tried except on Richards' solution agar where it is 27·5°C. There is no growth at 5·5°C. and 37·5°C. but at latter temperature growth occurs on Richards' solution agar.

9. The size and septation of spores are remarkably affected by temperature, the nature of the medium and relative humidity. The size and septation of spores are greatest on Richards' solution agar at 27·5°C; below 20°C. and above 30°C. the spores formed were smaller in size and fewer in septa. The spores formed on wheat straw were longest in size and with a large number of septa. The size and septation of spores increase with a rise in relative humidity.

10. Secondary spores are often formed in culture, these are either unseptate or one-septate. The optimum temperature for spore germination is 25°C. 100 per cent. carbon dioxide had a retarding effect on spore germination. The optimum hydrogen-ion concentration for spore germination is 5·9. There is no germination at pH 2·1-2·8 and 9·1.

11. The optimum hydrogen-ion concentration for the growth of the fungus is 7·1. There is no growth at pH 2·1-2·7 and 8·5.

12. Infection readily takes place in 72 hours with mycelium as inocula. With spores suspension, infection spots are formed after 5 days. It can also infect *Cajanus indicus*, *Glycine hispida*, *Vigna catjang*, *Phaseolus radiatus*, *Phaseolus mungo*, and *Phaseolus aconitifolius*.

13. During the course of study two saltants were obtained and compared with the parent form.

14. This fungus is in most virulent form in the months of February and March. A study of the meteorological records of Pusa from 1928-1932, revealed the fact that during those months, both temperature and relative humidity are most suitable for the spread of the fungus. The disease can successfully be controlled by growing disease-resistant early varieties.

In the end I like to express my deep sense of gratitude to Dr. W. McRae, M.A., D.Sc., F.L.C., the Director and the Imperial Mycologist, Pusa, for giving me all possible facilities for the investigation and to Dr. M. Mitra, M.Sc., Ph.D., D.I.C, the First Assistant, Mycological Section, Pusa, for the keen interest taken by him throughout the investigation.

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IX. Explanation of Plates XXXII-XXXIV.

Plate XXXII.

(The figures are reduced to 2/3.)

Fig. 1. Early stage in the infection of leaf; under surface. × 1½.

Fig. 2. Later stage of the same. × 1½.

Fig. 3. Advance stage in the infection of leaf under surface. × 1½.

Figs. 4 & 5. Enlarged young and old spots of leaf. × 8.

Figs. 6 & 7. Early and advanced stages in the infection of petiole × 1½.

Fig. 8 A spot of the same enlarged. × 1½.

Fig. 9. A portion of infected stem. × 1½.

Fig. 10. A spot of the same enlarged. × 6.

Fig. 11. Early stage in the infection of pod. × 1½.

Fig. 12. Later stage of the same. × 1½.

Fig. 13. A spot from pod enlarged. × 4.

Fig. 14. Early stage in the infection of seed. × 4.

Fig. 15. Later stage of the same. × 4.

PLATE XXXIII.

(The figures are reduced to 2-3.)

Fig. 1. A portion of transverse section of leaf showing stromata in the air space bearing a fascile of young conidiophores projecting out. × 800.

Fig. 2. A facile of mature conidiophores with young conidia attached to them. × 800.

Figs. 3, 4, & 5. Mature conidiophores with young conidia attached. × 800.

Fig. 6. A portion of branched conidiophore. × 800.

Figs. 7-12. Typical conidia in nature. × 1,850.

Figs. 15-18. Chlanydospores produced from sterilized *Dolichos Lablab* stems at 27·5°C. × 800.

Fig. 19. Conidiophore with an attached conidium from Richards' solution agar at 35°C. × 1,850.

Fig. 20. Conidiophore with conidia attached from Richards' solution agar at 10°C. × 1,850.

Fig. 21. Conidiophore with conidia attached from Richards' solution agar at 31°C. × 1,850.

Fig. 22. Conidiophore with conidia attached to it from Richards' solution agar at 35°C. × 1850.

Fig. 23. Same as in Fig. 22. at 25°C. × 1850.

Fig. 24. Conidiophore from Richards' solution agar at 31°C. × 800.

Figs. 25-32. Typical conidia from Richards' solution agar at 27·5°C. × 1,850.

Fig. 33. A typical conidium with two secondary conidia from Richards' solution agar. × 1,850.

PLATE XXXIV.

Fig. 1. Zonation in alternate light and darkness on thin plate of Coons' agar, one month old culture (Under surface of culture).

Fig. 2. Zonation due to fluctuating temperature (18°C.-31°C.) on thin plate of Coons' agar, fifteen days old culture (Under surface of culture).

Fig. 3. Zonation in alternate light and darkness at 100 per cent. humidity on Coons' agar, fifteen days old culture. Saltant D_1, present as a sector (Upper surface of culture).

Fig. 4. Appearance of a non-sporing type of saltant, white sector (D_1), on Coons' agar, eighteen days old culture at 18°C. (Upper surface of culture).

Fig. 5. Appearance of a non-sporing type of saltant (D_2) on Coons' agar, one month old culture at 31°C. (Under surface of culture).

Fig. 6. Appearance of a non-sporing type of sector (D_1), on Coons' agar fifteen days old culture at 30°C. (Under surface of culture).

THE LIFE-HISTORY OF A COMMON COCKROACH (*PERIPLANETA AMERICANA* LINNEUS)

BY

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(Received for publication on 13th December 1932)

(With Plates XXXV-XXXVIII)

INTRODUCTORY NOTE.

Our common House (and Ship-) Cockroach is too well-known in India to require any introduction, but its life-history under Indian conditions has remained something of a mystery. In spite of its abundance we did not know definitely how long it took to develop to the adult state or how many life-cycles were passed through annually. Many years ago several attempts were made in the Pusa Insectary to ascertain these facts but those attempts failed at that time, as this cockroach, in spite of its dominance under natural conditions, proved to be very difficult to rear under artificial conditions. Some five years ago Mr. Nigam took up this work again under my supervision and the present paper records his results. It represents a great deal of work under difficult conditions and many disappointments.

PUSA;
The 31st March 1932.

T. BAINBRIGGE FLETCHER,
Imperial Entomologist.

INTRODUCTION.

The common house cockroach of India is the one commonly called the American cockroach, and is technically known as *Periplaneta americana* L. Everyone in this country is only too familiar with this insect as it infests every dwelling house, in the store rooms, kitchens, cupboards, and libraries, etc. Although this insect is very common and familiar to every one, the details of its life-history are little known. This is because its life-cycle is fairly long, and, owing to its peculiar habits, observations cannot be made so easily as is generally supposed. Its rearing

in captivity is by no means easy, especially under conditions where everyday observations are possible.

The cockroach is structurally one of the most primitive insects. It has become a classic in that every student in zoology has to dissect it. In some countries this insect has been studied in its various aspects. The present paper is the result of observations on the life-history and bionomics of the insect under Indian conditions.

Early history and distribution.

The cockroach is one of the most primitive insects in the sense of its early appearance on the globe. The fossil remains of the roaches are abundantly met with in the coal formations, long before the more common features of insect life of the present day had begun to appear. The species now extant are few in comparison with the abundance of forms in the carboniferous age, which might with propriety be called the age of cockroaches. The moisture and warmth of that distant period appear to have been as favourable to the development of this order of insects as to the growth of the plants which provided the abundant raw material for coal formation.

The house cockroaches of today were undoubtedly very early associated with man in his primitive dwellings, and through commerce have been carried to all quarters of the globe. The American cockroach, *Periplaneta americana* L. which is the common house cockroach in this country, was originally a native of sub-tropical and tropical America. It has now become cosmopolitan in distribution.

In India it is found almost throughout the whole country. The other common species in this country is *Periplaneta australasiæ* Fabr. which is very similar to *P. americana* L., but it is not so widespread as the latter, being found mostly in Southern India and Ceylon. The two species can be readily distinguished by the presence of two yellow streaks on the costal margin of the fore-wings, running obliquely from the hind margin of the pronotum in the case of *P. australasiæ* Fabr.

Habits.

Adult cockroaches live in dark places, kitchens, under stones, and in cupboards, etc. They are omnivorous—little comes amiss to them. Paper, books, boots, hair, bread and fruits are all devoured by them with avidity. They display a partiality for sweetened food and are specially fond of starchy food. They are the source of great damage to clothes, and the bindings of the books in libraries and publishing houses. The paste or sizing used in cloth covers and in the binding of books is very attractive to them. They always scrape and disfigure the covers

of cloth-bound books. Their flattened body enables them to get entrance through very narrow cracks and crevices, and to take shelter almost anywhere. Heat, damp, and good food are the ideal conditions for their development and multiplication. They need water also. In the course of observations spreading over a series of years it was observed that both nymphs and adults were attracted to warmth and moisture. They congregate in damp places or where water is readily available. If the water provided in the breeding cages dried up, and was replenished after three or four days, the cockroaches were observed to run from all sides to the water dish. They usually move quickly and their speed of movement and flattened bodies render them difficult to catch or kill. The females, when bearing an egg capsule, are somewhat sluggish in their movements. They do not usually pass fæces till the capsules are laid. They love darkness. During the day or when a room is lighted they keep to the hiding places, but as soon as it is dark they come out. If the light is suddenly turned on, there is a great scamper and scurry to get away. They leave their excreta over anything, and thus spoil articles of food, which do not lose their unpleasant roachy odour even when cooked. Their omnivorous and unclean feeding, combined with their habit of cleaning their front tarsi and antennæ with their mouth when these have become soiled, render them a nidus for various pathogenic organisms which pass out of the body unharmed along with the excreta. Human food which is soiled either by cockroach fæces, or by the dirt casually borne on their feet, may thus be a cause of serious disease.

Predatory habit.

The cockroaches are frequently seen to eat their own kind. If they are hungry and no food is available inside the cage they attack and devour the weaker ones. The unprotected oötheca are often eaten up by them. A definite case of predaceousness was observed by Annandale [1910] in Calcutta. He found that on the evening of June 9th, during a heavy downpour of rain, numerous termites flew into his dining room, and were borne to the floor by the current set up by the electric fan. As they lay struggling many of them fell a prey to a lizard, while others were devoured by cockroaches (*P. americana* L.). Each cockroach stood over one of the termites with its legs spread out and firmly planted, and seizing the struggling insect with its jaws, began to gnaw the abdomen. If disturbed the cockroach carried the termite away in its mandibles. Sometimes the whole body except the wings was devoured, and sometimes only the abdomen.

Besides, cockroaches have been recorded to prey upon bed bugs in the houses. In this respect alone perhaps they may be said to be of some use to mankind.

The nymphs also, like the adults, are very active and run about with great agility. They also love darkness. They like starchy and soft food. They can

live without food for several days like the adults. In some cases when the food supply of the nymphs of the second instar was almost cut off for seven days they were all found living. Food with fungal growth is distasteful to them. The copious growth of fungus mycelia and spores inside the small glass tubes containing the nymphs did not prove fatal to them for a few days, but if they were kept there for a long time, they would die. The nymphs devour their own exuvia with avidity, and sometimes it becomes very difficult to find any trace of the exuvium. The older the nymphs the more quickly do they finish off their cast skins. Almost all the parts of the exuvium are eaten up except the leg moults. The third and the fourth exuvia were eaten away so quickly that at times it was found very difficult to detect the ecdyses.

Life-cycle.

Technique.—Several couples of *P. americana* L. were placed in large glass cages for copulation and oviposition. The egg capsules that were laid were removed, labelled, and kept separately for hatching. The nymphs which emerged from the capsules were kept each in a separate glass tube to note their growth and ecdyses. When they had grown big enough they were transferred into bigger cardboard or wooden boxes with glass tops. Some broods of the nymphs which hatched out of the same capsule were placed together in a single large glass cage with darkened sides and a layer of earth at the bottom. A regular supply of food and water was maintained in all the cages.

Formation and retention of the oötheca by the females.

Eggs of *P. americana* L. are not laid singly as in many other insects, but are brought together within the abdomen into a capsule or oötheca which is seen protruding from the genital opening of the females. When the formation of the capsule begins, its colour is whitish at the tip, which protrudes only slightly from the opening. Later on, with further formation of the capsule, it protrudes more and more and its colour changes to brown. When completely formed it is retained by the female for some time and deposited in a favourable spot. The period of retention of the capsule after it becomes visible up to the time when it is laid ranges from 6 hours to 25 hours. In general it ranges between 6 and 12 hours. The maximum period of retention actually met with did not exceed 25 hours as is shown in the following table. Haber [1920] has mentioned that oötheca are retained by the females until places of favourable moisture and thermal conditions are found. Thus, if a cage is too cold, too damp, or too well-lighted or too poorly provided with suitable material for egg-laying or for concealing the oötheca, the female may retain the oötheca for several days. In cages with fovourable environment the oötheca were deposited the day after protrusion.

TABLE I.

Showing the period of retention of oöthecæ by the females.

Mother seen with protruding capsule	Date of the laying of the capsule
1927	1927
7th May (Morning)	7th May (Night)
11th May ,,	11th May (3 p m.)
12th May ,,	12th May (5 p.m.)
13th May (Noon)	13th May (10 p.m.)
14th May ,,	15th May (Evening)
16th May (Morning)	17th May (Midnight)
16th May ,,	16th May (10 a.m.)
17th May ,,	17th May (Evening)
17th May ,,	18th May (2-5 p.m.)
18th May ,,	18th May (Night)
18th May ,,	19th May (Noon)
20th May ,,	20th May (5 p.m.)
20th May (Noon)	21st May (5 a.m.)
23rd May (Morning)	24th May
26th May	26th May (Night)
30th May (Morning)	30th May ,,
31st May	1st June ,,
4th June	5th June ,,
7th June (Morning)	7th June (Evening)
14th June ,,	14th June (Night)
15th June ,,	15th June ,,

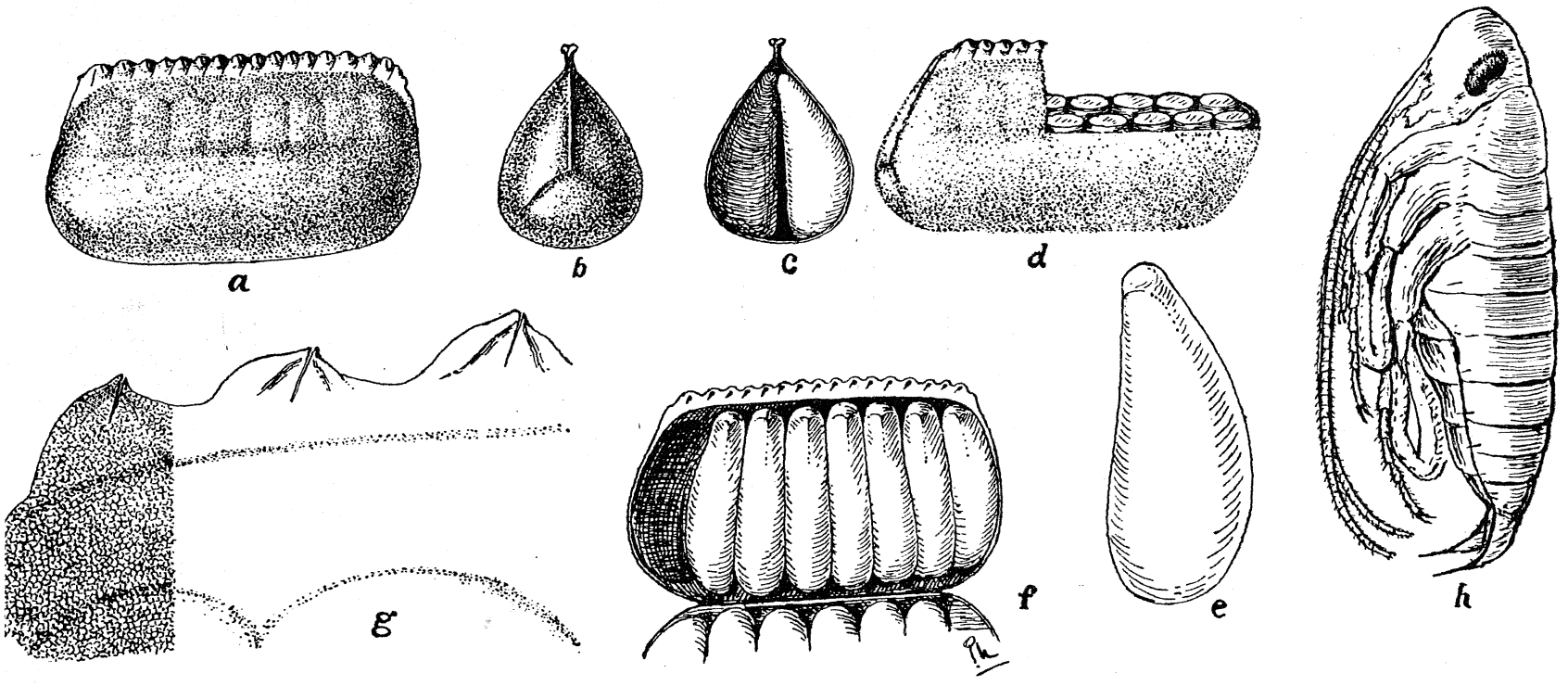
SEASON OF EGG DEPOSITION.

The capsules are generally laid during the summer, beginning from April or May. The breeding, which is very vigorous in summer, slows down with the approach of winter months. Very few, if any, capsules are laid in winter. The activity of the adults as well as of the nymphs becomes dormant during the winter, as is generally the case with insects. With the advent of summer the egg-laying restarts.

EGG DEPOSITION.

A female is mature and able to reproduce in 1-2 weeks after the final moult. According to Cottam [1922] also the newly emerged female is mature to lay eggs in 10-17 days after becoming adult. The laying of the egg capsule usually starts in 3-7 days after copulation. Fresh mating is not essential for the laying of each capsule. A female is able to lay several capsules even after the male is removed from the cage. Haber [1919] has recorded that as a result of single mating 13 egg masses were laid in 4 months.

The capsules are deposited by the females usually in the corners of the cage, or in some secluded and concealed spots, under bits of paper on other materials. The female, just before depositing the capsule, roughened the surface of the card-board which was placed in the cage. It gnawed the upper surface of the card-



(*a*) Lateral view of an Oötheca × 6.
(*b*) Front end of an Oötheca showing the marks of the genital armature × 6.
(*c*) Cross section of an Oötheca showing the position of the two rows of eggs and the slit × 6.
(*d*) Oötheca showing the alternate arrangement of the eggs × 6.
(*e*) An egg taken out of the Oötheca × 6.
(*f*) Oötheca showing the number and the arrangement of eggs × 6.
(*g*) Ridge of an Oötheca highly magnified showing the structure of the teeth and the sculpture on the shell.
(*h*) Pronymph × 24.

board until it made an appreciable groove. It did not drop the scrapings of the cardboard, but worked them up with the saliva from the mouth into a plastic material, which was stuck to the surface of the groove. After a short time the female was seen searching for the groove with its bent abdomen carrying the capsule. On locating the spot it laid the capsule in it.

The egg capsules, when laid, are generally covered up with bits of paper, rags or bread in order to conceal them. If nothing is handy then the female is seen utilizing the dried excreta of the cockroaches to conceal the capsules. Egg capsules which are deposited in unprotected spots and lie exposed are usually eaten up by other cockroaches or even by the mother. Very often the oöthecæ are seen attached, in their natural habitats, to rafters, and covered with bits of wood fibres chewed off by the strong mandibles of the mother. Sometimes, when no material is available inside the cage, the oöthecæ are dropped haphazard, and remain uncovered.

The number of egg-capsules laid by a single female during her life-time is variable. It depends upon the opportunities for mating, the temperature, the food supply and the longevity. The females which were isolated and had no access to males were found to lay either no capsules or only one or two capsules of smaller size. These did not hatch. One female was kept alone in one cage for 8 months from October to May without laying any capsule. The usual number of egg-capsules laid by a single female was found to be 10-15 within ten months from September to June, out of which the period of about 5 winter months (from November to March) was passed without any egg-laying. It is very probable that the number of capsules laid in their natural habitats may be much higher than the number laid in captivity. Illingworth [1915] has recorded that in the case of *Phyllodromia hespes* Perk., the maximum number of egg-capsules deposited by a single female was 38. The interval between successive egg-laying is on an average found to be 4-10 days when the females have ample opportunities for mating. The maximum interval met with during the course of observations was 18 days and the minimum was 2 days. Haber [1919] found that a female laid 13 egg masses at an interval of 5-12 days in 4 months.

Egg-capsule (*Plate XXXV, figs. a-g*).—The capsule or oötheca is a hard chitinous body, bean-shaped, and of a brown colour when freshly laid. The colour changes to dark brown or even black in about 24-48 hours. The capsule is more or less oval in cross-section (Plate XXXV, fig. c). The length of the capsule along the upper ridge is 7·5 mm. and along the ventral or opposite side it is 8·5 mm. The breadth or thickness is 4·5 mm. and the height is 5 mm. Some of the capsules were found to vary in size and measured only 6·5 mm. along the upper ridge.

Ridge (*Plate XXXV, figs. a and g*).—The upper side of the capsule bears a conspicuous ridge which is serrate. It bears usually 16 teeth. The apices of the teeth are darker in colour than the rest of the body of the capsule. The space just below the projecting teeth is light brown in colour. Each tooth when seen under high magnification discloses a minute opening at the apex (Plate XXXV, fig. *g*). The ridge is bordered below by a dark line running parallel to the upper edge. The portion of the capsule below the ridge contains the eggs whose upper outline is marked off as depressions on the chitinous covering. The upper one-third of the body of the capsule is narrower than the lower two-thirds, where the basal portions of the eggs are lodged (Plate XXXV, figs. *c* and *f*).

Egg (*Plate XXXV, figs. c-f*).—The eggs are arranged inside the capsule in two longitudinal rows, so that the individual eggs of the two rows alternate (Plate XXXV, fig. *d*). The usual number of the eggs met with is 16, although in a few cases a less number is found. In a transverse section of the capsule we find that there is an open slit between the eggs of the two rows and this runs along the whole length of the capsule, showing thereby that the eggs of one row, although alternating with those of the other, are not wedged in between them. Each egg is elongate and cigar-shaped, slightly bent toward the upper or head-end. It is wider on the lower or basal end. The head-end lies opposed to the ridge. The egg is creamy yellow in colour and its outer covering is very thin, transparent, and membranous.

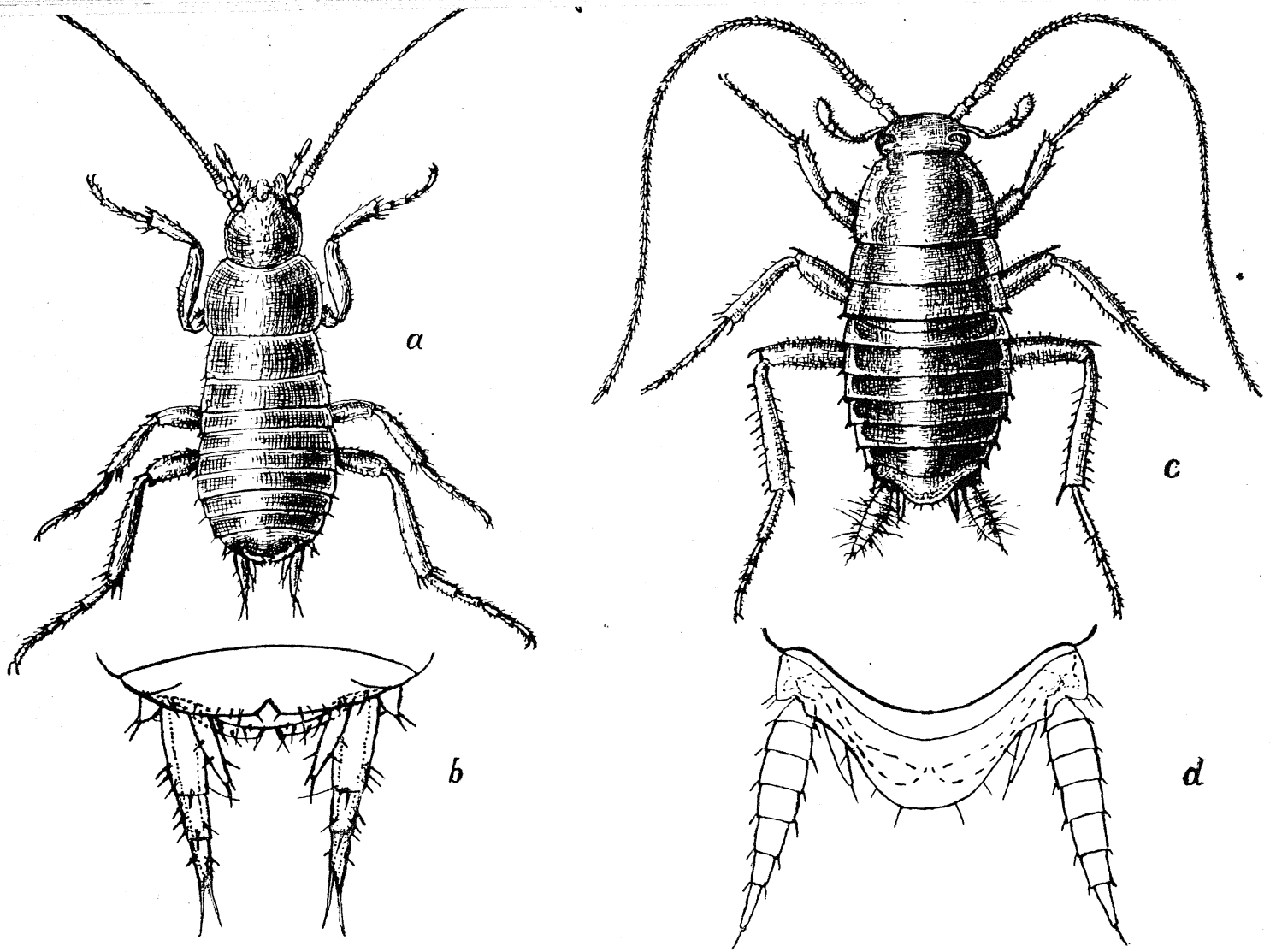
Hatching of the eggs.

The egg masses laid by the females were kept in moist and warm cages, and numbered. They were found to hatch almost invariably about 27 or 28 days after the date on which they were laid. This period of 27 or 28 days was remarkably constant, with very rare variations, when it was lengthened by 2 or 3 days only. Haber [1919] has stated that in Minnesota the egg capsules take about 70 days to hatch at a temperature of 77 degrees in dark cages. Table II illustrates the period of hatching.

Table II.

Showing the period of the hatching of capsules.

Date of laying of capsules	Date of hatching of the capsules	Number of days
May 1927	June 1927	
16th	12th	27
17th (Night)	13th	27
17th (Evening)	14th	28
18th (2 p.m.)	14th	27
19th (Noon)	15th	27
20th (5 p.m.)	16th	27
21st (5 a.m.)	18th	28
26th (Night)	22nd	27



(*a*) Dorsal view of nymph of the first instar × 12 (from slide). (*b*) Dorsal view of the tip of the abdomen of the first instar × 36. (*c*) Dorsal view of nymph of the second instar × 12 (from slide). (*d*) Dorsal view of the tip of the abdomen of nymph of the second instar × 36.

When the nymphs are about to emerge, the ridge of the capsule dehisces longitudinally and nymphs make their appearance by the head-end. The anterior portion is first protruded, then the lateral movements are frequent until the body is clear out of the egg-shell. The amniotic covering remains attached to the mouth of the egg-shell as a white fringe. Maternal care for the nymphs is absent in the case of this insect.

The number of nymphs which hatch out of a capsule is 16 as a rule. The number of eggs found inside the capsule is also 16. Some capsules are smaller in size and the number of nymphs emerging out of them is also less. Laing [1921] has mentioned that the maximum number of eggs in a capsule of *P. americana* L., is 14, whereas the writer has met with 16 as the maximum number in this country. Haber [1919] has stated that in Minnesota the egg-cases of this species may contain 18-28 eggs and from 20-24 are common.

Nymphs.—first instar (*Plate XXXVI, fig. a*).—When the nymph emerges from the egg it is pale or cream coloured with dark ochraceous prominent eyes. With the lapse of time and exposure to air the body assumes a darker colour. It begins to move about soon after emergence. The nymphs are very much like the adults excepting that they are devoid of wings, and are smaller in size. The sub-anal styles are present in the nymphal stages of both the sexes, though these are absent in the adult females.

The first moult occurs after about a week from the date of hatching. In most cases the nymphs were observed to eat their own exuvia. The nymphs of *P. americana* L., differ in this respect from *P. orientalis* Sulz., in which Cornelius [1853] states that the first moult occurs just when the nymph escapes out of the capsule. Table III gives the measurements of a newly emerged nymph.

TABLE III.

Showing measurements of nymphs 12-20 hrs. after hatching.

No.	Length of body excluding cerci mm.	Length of cerci mm.	Segment of cerci mm.	Length of antenna mm.	Length of pronotum mm.	Length of 1st and 2nd thoracic terga mm.
1	4·1	0·9	3	4·5	0·75	0·78
2	4·2	0·9	3	6·5	1	1

Second instar (*Plate XXXVI, fig. c*).—Immediately after the first ecdysis the colour of the chitin is more or less pale yellow. This, however, darkens with exposure to air. There is a small black fascia on the mid-abdomen dorsally and it

is seen through the transparent chitin on the dorsum. This, later on, elongates and forms a longitudinal band extending from the posterior end of the pronotum up to the tip of the abdomen. Other than an increase in size in the thoracic region there is no appreciable external change in the body from that of the first instar. Only the size increases appreciably and the cerci become more segmented. The measurements are :

TABLE IV.

Nymphs of second instar.

Length of the body mm.	Length of the cerci mm.	Segments of cerci mm.	Length of the antenna mm.	Length of the pronotum mm.	Breadth of the body mm.	Length of 1st and 2nd thoracic terga mm.
4·5	1	7	6	1·4	2	1·25
4·5	1	7	5·5	1·7	2	1·25

The nymph, when about to moult, becomes stationary. There appears a longitudinal slit on the thorax dorsally and the nymph gradually draws itself through it. The period of the duration of the second instar is much longer than that of the first instar. On an average it varies from 3 to 4 weeks. In the first instar it was noticed that almost all the nymphs from the same capsule cast off their skin more or less simultaneously or within a space of 2 or 3 days. But in the case of the nymphs of the second instar the moulting is not simultaneous. It takes place within an interval of 7 or 8 days. In one case, however, it was noticed that the second moult, instead of occurring within 3 to 4 weeks after the first moult, did not occur till about 8 weeks after the first moult.

Later instars (*Plates XXXVII and XXXVIII*).—It may be said, in general, that nymphs do not undergo any marked change externally while passing through subsequent instars, except that the size increases at each moult. A change in the colouration of the pronotum is noticeable in the sixth instar. The pronotum, which is unicolourous in the earlier stages, develops two white patches on each side of the dorso-median line. In the penultimate stage, the nymphs are fairly big in size and the wing bases become especially enlarged (Plate XXXVIII, fig. *a*). After the last moult the insect comes out with fully developed wings, which are soft and transparent in the beginning. Just after the moult the whole insect looks white with black and prominent eyes. After exposure for some time the wings become brown in colour and hard and tough in texture. The colour of the body of the insect changes, as usual, to dark brown after a few hours.

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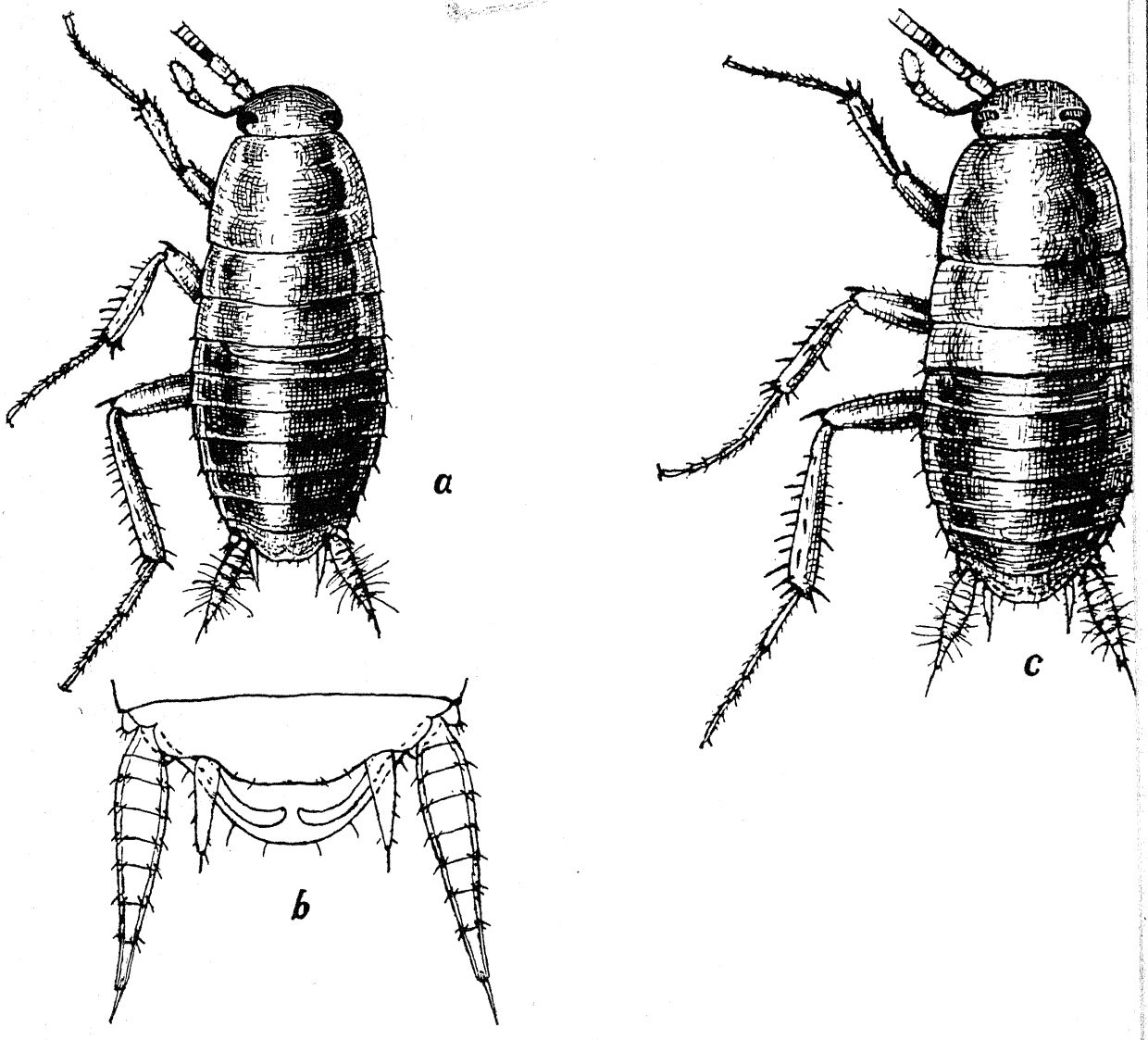
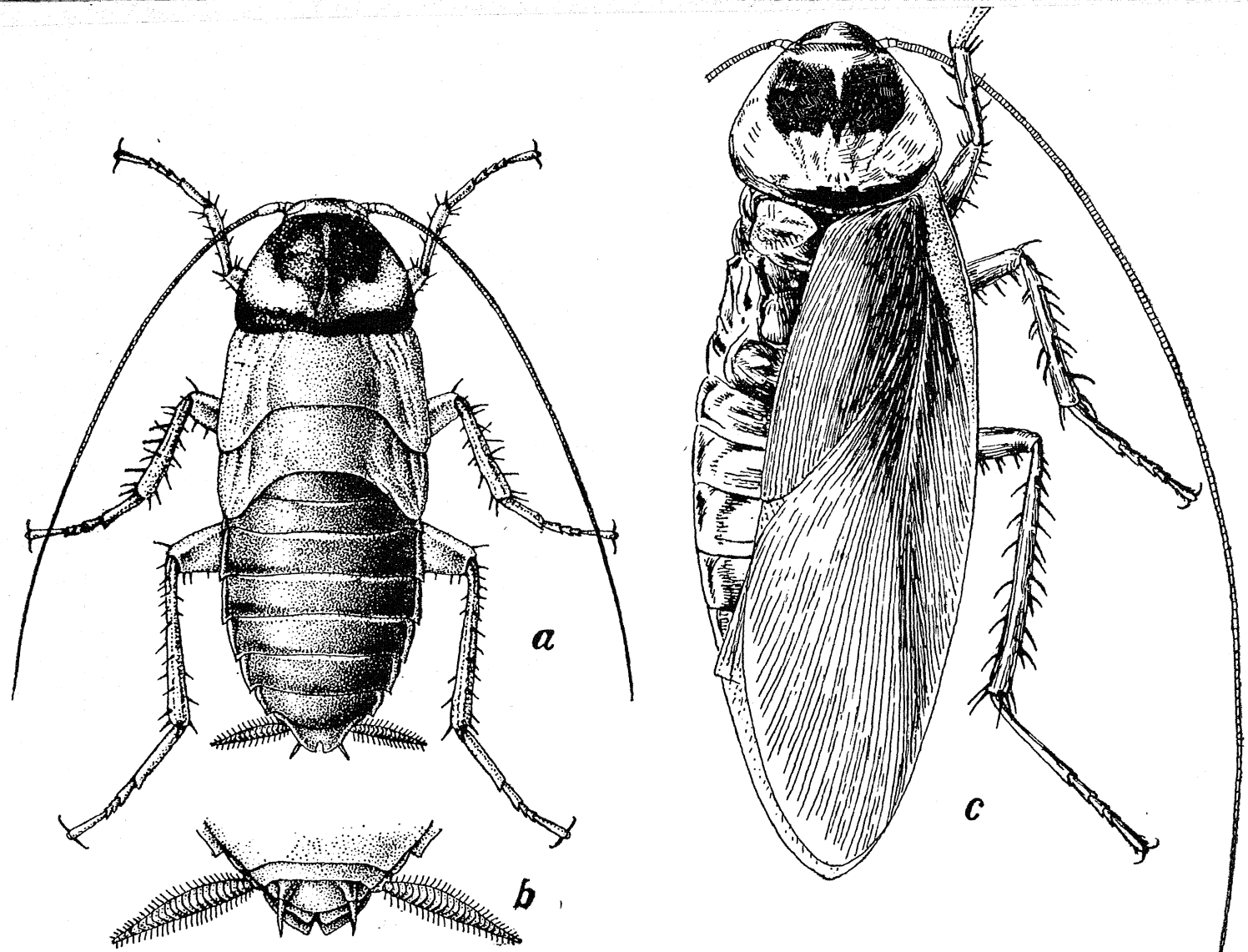


(*a*) Dorsal view of nymph of the third instar ×12. (*b*) Ventral view of the tip of the abdomen of nymph of the third instar × 36. (*c*) Dorsal view of nymph of the fourth instar × 12.



(*a*) Penultimate stage of the nymph. Dorsal view × 3. (*b*) Ventral view of the tip of the abdomen of the penultimate stage of male × 6.
(*c*) Adult stage of male. Dorsal view × 3.

The following tables give an idea of the duration of various instars through which the nymphs pass before attaining the adult stage.

NUMBERS AND DURATION OF INSTARS.

TABLE V.

Instar of the nymph hatched on 1st August 1928.

Number of the moults	Date of moulting	Duration of the instars (days)
1st	12th August 1928	12
2nd	26th „ „	14
3rd	2nd September 1928	7
4th	17th January 1929	137 (4½ months)
5th	8th February „	22
6th	25th August „	198 (6½ months)
7th (Adult)	20th September „	26
	Total	13 months and 20 days

TABLE VI.

Instars of the nymph hatched on 1st August 1928.

No. of moults	Dates of moultings	Duration of the instars (days)
1st	12th August 1928	12
2nd	22nd August „	10
3rd	6th September 1928	15
4th	11th October 1928	36
5th	24th „ „	13
6th	18th March 1929	145
7th	5th April „	18
8th	13th August 1929	129
9th (Adult)	5th September 1929	23
	Total	13 months and 5 days

TABLE VII.

Instars of the nymph hatched on 8th August 1928.

Number of moults	Dates of moultings	Duration of instars (days)
1st	15th August 1928	7
2nd	27th „ „	12
3rd	?	?
4th	26th September 1928	
5th	23rd March 1929	177
6th	8th June „	77
7th (Adult)	2nd September 1929	86
	Total	12 months and 24 days

From the above it will be seen that the number of moults is variable. Marlatt [1919] also mentions that cockroaches undergo a variable number of moults, sometimes as many as seven. Generally speaking, there appear to be about 7 or 8 moults. The duration of the instars is very variable. The earlier instars are of a shorter duration than the later ones. The instar which falls during the cold months is exceptionally long. In winter there appears to be a partial hibernation.

The development of this insect is unquestionably slow, and the period of attaining the adult stage is also variable, depending upon favourable climatic conditions and the supply of food. In captivity and under artificial and adverse conditions the development seems to be slower than under favourable conditions. The nymphs that were kept singly in glass cages took about 13-15 months or so to become adults, whereas some of the nymphs, that were kept together in big cages with a layer of earth at the bottom, with darkened sides, and with enough food and moisture, became adults in ten months or so. Thus from a number of data obtained while working out the bionomics of this insect it can safely be said that there is practically only one cycle in a year under conditions at Pusa. Table VIII shows the actual periods taken by various nymphs from the egg to the adult stage.

TABLE VIII.

Showing the nymphal periods.

Number of the nymph	Date of hatching	Date of becoming adult	Total nymphal period	
			Months	days
A	1st October 1928	12th August 1929	10	12
B	Do.	17th August 1929	10	17
C	Do.	Do.	10	17
D	Do.	1st October 1929	12	0
E	Do.	21st March 1930	18	21
F	Do.	22nd March 1930	18	22
G	Do.	Do.	18	22
H	Do.	15th April 1930	19	15
I	Do.	2nd May 1930	20	11
J	Do.	1st-7th June 1930	21	0
O_{11}	1st August 1928	20th September 1929	13	20
O_{14}	Do.	5th September 1929	13	5
P_{10}	8th August 1928	2nd September 1929	12	25
P_{32}	Do.	21st March 1930	19	13

From the above it will be apparent that the total period covered by nymphs to become adults ranges between 10-21 months. This wide range of the period shows that the development depends upon favourable conditions. Marlatt [1908] has recorded that the total nymphal period of *P. americana* is 11 months. He points out that the rate of growth depends upon food and temperature and that the period is often lengthened under unfavourable circumstances.

The growth of the insect in size is indicated in Table IX.

TABLE IX.

Showing measurements of the nymphal and adult stages of P. americana.

Stage	Length of the body mm.	Breadth of the body mm.	Length of antenna mm.	Length of pronotum mm.	Length of wing bases of 1st and 2nd thoracic terga mm.	Length of cerci mm.	Segments of cerci
3rd instar . .	10·5	3·7	20	1 2	1·2	1·25	9
Penultimate stage	30	11	31	6·7	7·5	3·2	18
Adult stage No. I	35·6	12·7	45·72	8·8	..	6·5	20
Adult stage No. II	38	12·7	38·1	11·2	..	6·5	20

The dimensions of the nymphs of a particular stage as well as of the adults are not always uniform. They are found varying in many cases. All the adults of the same sex do not measure equally. It appears that the size depends upon the environment and food supply.

Adult stage.

The adult cockroach, which emerges after the last ecdysis, is fully winged. The first pair of wings or the elytra in the males are long and extend beyond the abdomen, whereas in the females they are as long as the abdomen, or extend only a little beyond it. After the last ecdysis the adult is flat, light ferruginous in colour, with head deflexed. Dorsally the head is black with reddish blotches below the antennæ. The antennæ are longer than the body. The pronotum is sub-rotundate, yellowish testaceous, with two large whitish spots on the dorsum medially with indistinct outline. The supra-anal lamina of the male is broad and incised ; lobes are broad and rounded. The supra-anal lamina of the female is triangular, deeply and narrowly incised ; the lobes are narrow and the apex obtuse. The cerci are more than twice as long as the laminæ. Styles are present in the males only.

Regeneration.

The phenomenon of regeneration is well illustrated by this species of cockroach. These insects possess long legs and long antennæ, which are very liable to breakage. Like many other insects they have the power to regenerate the lost parts, provided the final adult stage has not been reached, and provided that the stump of the broken organ remains. If a limb is entirely destroyed, regeneration is not possible. One antenna of a nymph was broken during measurement and drawing. It was found regenerated after the next moult.

Cockroaches and disease.

It has long been suspected that cockroaches, if not the actual carriers of pathogenic organisms, are at least likely to serve as a nidus, on account of their peculiar habits and habitats, for the existence, development and ultimate dissemination of pernicious organisms. No critical work seems, hitherto, to have been done in this country to ascertain the rôle played by these ubiquitous insects in the propagation and dissemination of disease germs. From what has been done in the past these insects have been known to be responsible for causing the souring of milk with their intestinal bacilli. Barbar [1912] has shown them capable of disseminating plague and cholera bacilli, which multiply in their intestines without losing their virulence, the hosts themselves remaining immune.

Macfie [1922] has shown that the following organisms pass through the intestines of *P. americana* L., quite unharmed.

Bacillus tuberculosis, *B. leprœ*, cysts of *Entamœba histolytica*, *E. coli*, cysts of *Giardia intestinalis*, eggs of *Ancyclostoma duodenale*, *A. ceylonicus*, *Necator americanus*, *Ascaris lumbricoides*, *Trichuris trichura*, *Tœnia saginata*, and *Schistosoma hœmatobium*.

Miall and Denny [1886] have mentioned the presence of the following in the body of the cockroach :—

Amœba blattœ Butshl., *Gregarina blattarum* Siele, *Lophomonas blattarum* Stein, and *Filaria rhytipleuritis*, etc., etc.

It rests with those more qualified in this line to extend the field of knowledge regarding the rôle of this insect in acting as a reservoir for the development of pathogenic bacteria which are causative of ailments, which lay a heavy toll of life annually. In this paper the facts regarding the bionomics of the insect as observed under local conditions have been set forth.

ACKNOWLEDGMENT.

In the end I have to acknowledge that I am deeply indebted to my Chief, Mr. T. Bainbrigge Fletcher, R.N., F.L.S., F.E.S., F.Z.S., for the help and guidance that I have received during the course of work.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS.*

No. 12.—ANALYSIS OF VARIETAL TESTS WITH WHEAT CONDUCTED AT SAKRAND, SIND, 1931-32.

BY

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(Received for publication on 3rd October 1932).

Mr. K. I. Thadani, of the Department of Botany in Sind, Sakrand, conducted a varietal trial with 5 varieties of wheat in the Agricultural Research Station, Sakrand. The experiment was laid out on an area of 2 acres, divided into two blocks of one acre each. Each block was divided into 20 plots of 1/20 acre, so that the five varieties were replicated 4 times in each. The lay-out is shown in Fig. 1. Mr. Thadani stated that the plots were randomized. On the assumption of a random sample we may proceed with the analysis.

E	81·0	A	76·2	B	81·3	C	81·8
D	59·5	E	77·7	A	71·9	B	66·1
C	44·1	D	63·4	E	74·3	A	66·6
B	81·2	C	74·7	D	83·3	E	89·2
A	88·0	B	68·0	C	46·6	D	78·2
D	78·9	E	77·3	A	89·0	B	85·2
E	79·8	A	73·3	B	73·3	C	61·4
A	76·2	B	74·9	C	66·7	D	63·4
B	83·3	C	76·8	D	76·4	E	85·9
C	78·5	D	68·9	E	95·1	A	72·5

Fig. 1.

* A large number of enquiries of a statistical nature are being received from agricultural workers in different parts of India. Many of these enquiries are of considerable general interest, and it is proposed to publish notes on selected topics from time to time. These notes will deal mainly with statistical methods and procedure, and it is not intended that they should always contain new matter. [Ed.]

The average area harvested for each plot after excluding border rows and bunds was 1,555 sq. feet (1/36 acre). The yield of wheat in lbs. per plot of 1,555 sq. feet is shown in Fig. 1.

Mr. Thadani noted that in Block II, there were "*kalar* patches," the area and exact location of which are given in the following table :—

Location			Area of the patch (Sq. feet)
Block	Column	Row	
II	4	4	139
II	4	5	85
II	3	4	74
II	2	4	40

The harvested area included these patches. A glance at Fig. 1 does not suggest that the yield-figures are seriously affected by these patches. The detailed analysis also supports the view that the "patches" may be neglected without introducing appreciable errors.

We may analyse the resuls separately for the two blocks. The analysis of variance for Block I is given in the next table.

—	D. F.	Sum of squares	Mean square	Value of z	
				Observed	5 per cent.
Varieties	4	790·27	197·57	0·0932	0·5907
Columns	3	110·91	36·94		
Residual	12	1967·38	163·95		
Columns and residual	15	2078·29	138·55		
Total	19	2868·56			

The observed and 5 per cent. expected values of z are also given in the same table. The observed value of z is much below the 5 per cent. level, so that the varietal differences do not appear to be significant.

The mean yields of the different varieties * are shown in the following tabel: —

Varieties	Mean yield in	
	Lbs. per plot	Percentage
A	75·68	104·17
B	74·15	102·05
C	61·80	85·06
D	71·10	97·86
E	80·55	110·86
Mean	72·66	100·00
S. E.	6·40	8·81

A similar analysis for Block II is shown in the next two tables.

—	D. F.	Sum of squares	Mean square	Value of z	
				Observed	5 per cent.
Varieties	4	471·76	117·94	0·3634	0·5907
Column	3	137·38	45·79		
Residual	12	684·25	57·02		
Column and residual	15	821·63	54·77		
		1293·39			

The value of z is below the 5 per cent. level of significance, so that the varietal differences again appear to be insignificant.

* The names of the varieties tested were :—
A=Pusa 12, B=Pusa 80/5, C=Pusa 111, D=Pusa 114, E=Cph 47.

The mean yields in Block II are shown below.

Varieties	Mean yield in	
	Lbs. per plot	Percentage
A	77·75	101·18
B	79·18	103·05
C	70·85	92·20
D	71·90	93·57
E	84·53	110·01
Mean	76·84	100·00
S. E.	3·78	4·92

The similarity of the results yielded by a separate analysis of the two blocks suggests that it might be desirable to analyse the data for both blocks taken together. This will give us eight replications which are likely to lead to an increased precision of comparison. The analysis is shown below.

—	D. F.	Sum of squares	Mean square	Value of z	
				Observed	Expected 5 per cent.
Varieties	4	1182·63	295·66	·6009	·4992
Block	1	177·64	177·64		
Column	6	248·29	41·38		
Residual	28	2729·79	97·49		
Columns and residual	34	2978·08	87·59		
	39	4338·35			

The observed value of z now lies between the one per cent. and the 5 per cent. point. That is, such a value of z is likely to occur less than once in 20 trials. We may, therefore, consider the varietal differences to be significant. The mean yields are shown in the next table.

Varieties	Mean yield in	
	Lbs. per plot	Percentage
A	76·71	102·62
B	76·67	102·58
C	66·32	88·73
D	71·50	96·65
E	82·54	110·42
Mean	74·75	100·00
S. E.	3·49	4·67

We conclude :—

(1) There is no significant difference in yield between A and B or between C and D.

(2) Varieties A and B are significantly superior to C but not to D.

(3) Variety E is significantly superior to Varieties C and D.

The experimental error is of the order of 5 per cent., and hence the critical difference (which may be considered significant) is of the order of 15 per cent.

It will be noticed that the division of the experiment into columns has been totally useless, for the columnar variances are in all three cases actually lower than the corresponding residual variances. This justifies the inclusion in the analysis of the plots with "*kalar* patches".

Full details of the experimental results with a discussion of their agricultural significance will be published by Mr. Thadani in due course. The object of the present note is to illustrate the method of analysis.

Summary.

Results of varietal experiment with 5 strains of wheat in 8 replications conducted in Sakrand, Sind, have been analysed in this paper. Although the division of the field into eight blocks did not lead to any reduction in the fluctuations due to systematic changes in soil-fertility, the precision of the comparison was 4·67 per cent. It is interesting to observe that the presence of 4 *kalar* patches within the experimental area did not affect the yield of these plots materially.

No. 13—ON THE NEED OF RANDOMIZATION OF PLOTS IN FIELD TRIALS.

BY

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(Received for publication on 3rd October 1932.)

1. In the report of a varietal trial which reached us recently the plots were arranged as shown below, the variates p, q, r, s, t occurring as indicated in the chart. The author stated that the plots were randomized. I shall consider this point in some detail.

p	s	q	t	r	q	p	s
q	t	r	p	t	r	q	t
r	p	s	q	p	s	r	p
s	q	t	r	q	t	s	q
t	r	p	s	r	p	t	r

2. It will be noticed that the sequence p, q, r, s, t recurs regularly in all eight columns. In the present example there are 40 plots divided into 8 columns of 5 plots each, and it is required to distribute 5 varieties in such a way that each variety will occur once in each column. Five varieties can be distributed into 5 plots in 5. 4. 3. 2. 1. or 5 ! (factorial 5)* different ways. As the distribution in each column is independent of the distribution in any other column, the total number

* Factorial n (usually written as $n!$) stands for the continued product from 1 to n, *i.e.*, $1.2.3....(n-1).n$.

of ways in which all the 40 plots can be arranged is given by $(5!)^8$ or 42,998,169,600,000,000. In case the sequence p, q, r, s, t is kept intact within each column, the number of ways in which a column can be arranged is only 5. Hence for all eight colums the total number of ways would be $(5)^8$ or only 390,625 in this case.

Thus such systematic arrangements (in which the columnar sequence is kept intact) form only a small portion of all possible ways of arranging the plots. The actual proportion in the present example is $(5)^8/(5!)^8$ or $1/(4!)^8$ or 1 in 110,075,314,176.

We have seen that there are altogether $(5!)^8$ possible patterns. In a random selection no discrimination will be made between all these different patterns. In other words, the essential requirement of a random selection is that all patterns are given an equal chance of being selected, so that in the long run each pattern will occur an equal number of times. Thus, if we ensure the conditions of random sampling, an arrangement in which the columnar sequence is kept intact should occur only once in 110,075,314,176 drawings. Therefore, it appears almost certain that the present pattern did not occur by a process of random selection, but was arrived at by design.

3. The theory of error either in its classical form for large samples or in its more recent developments for small samples (as used in Fisher's t- and z- tests) is based on the assumption of random samples. Statistical tests can, therefore, be only applied when the sample is a random one. In fact, if the sample is not random such tests are wholly invalid. This essential theoretical requirement makes it absolutely necessary to have the plots properly randomized in order that statistical tests may be applied legitimately.

One word of caution is required. The fact that any pattern is systematic in character is not a sufficient ground for its rejection. The essential point is whether such a pattern is obtained by random selection or by some kind of (conscious or unconscious) design. It should be remembered that systematic arrangements also must occur sometimes in random selections. It is true that they occur very rarely (because they form only a very small portion of the total possible patterns), but nevertheless they do occur. And if we are certain that no bias is introduced in the process of selection, that is, if the conditions of random sampling are satisfied, then we have no right to reject a pattern however systematic or regular it may be. In fact such rejection would also invalidate the assumption of a random selection, and hence render invalid the application of statistical tests.

4. An illustration may make this point clear. Suppose we want to find out the average height of college students of age 20 studying in Calcutta colleges. Further suppose that it is not possible to take measurements of all the students.

We must then adopt the method of random sampling, and measure say 5 students in each class. After measuring several batches (or samples) of 5, we find that the average height is say approximately 5 ft. 6 in. Let us imagine that we now obtain a batch of 5 students all of whom are over 6 feet in height. The sample clearly is highly abnormal, and such a sample will occur very rarely. But we have no right to reject it, provided we are satisfied that it is a random sample. In fact the rejection of such a sample (however abnormal it may appear to be) would reduce the accuracy of our final estimate. The essential point here is, of course, the randomness of the sample, and not whether it is abnormal in character. If the condition of random sampling is satisfied then we feel sure that on taking a large number of random samples all heights will be represented in proportion to their respective frequencies in the population. If a sample of 5 students over 6 ft. in height occurs once in a while, it is certain that a sample of 5 students all under, say, 5 feet will also occur sometimes by chance, and it is the inclusion of both which will ultimately lead to the correct estimate.

5. We adopt an analogous procedure in using statistical theory for the interpretation of experimental results. The answer is invariably given in terms of the probability of occurrence of the observed result. For example, in a particular experiment we may find that the observed difference will occur only once in 5 (or 20, or 100, or 1,000 as the case may be) trials, the essential assumption being, of course, that all such trials are entirely unbiassed or random in character. Even when the probability is less than 1 in 1,000, or 1 in a million, we cannot be absolutely certain that the difference is real. We are, therefore, obliged to adopt a rule of interpretation (which is logically arbitrary in the sense that it depends on our own choice, and is not determined by the facts of observation); we usually adopt the rule that whenever the probability is 1 in 100 or less (or 1 in 20 or less) we shall consider the observed difference to be real. It will be observed that by adopting this rule we cannot insure that we shall be right every time, in fact we are bound to be wrong in about one per cent. (or 5 per cent.) cases. We do insure, however, that we are likely to be right, on an average in 99 per cent. (or 95 per cent.) of the cases considered by us. But the entire argument rests on the assumption of random samples, and the point to be emphasized here is that unless the condition of random sampling is strictly enforced, the adoption of an one per cent. (or one in a million) level of significance will be of no avail.

THE INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.), THE ITALIAN MILLET.

PART IV. SPIKELET-TIPPED BRISTLES.

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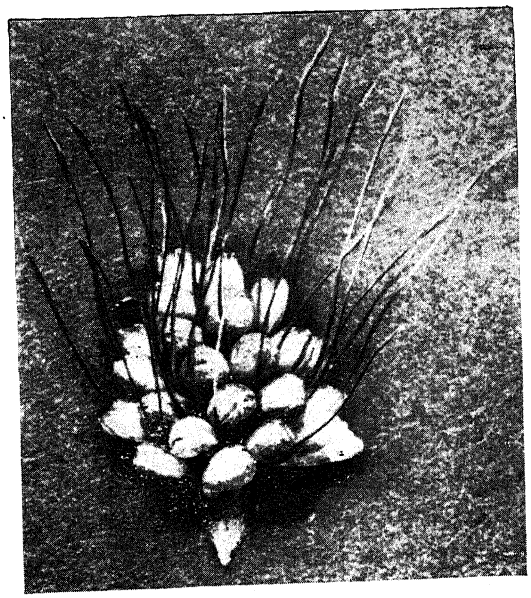
(With Plate XXXIX.)

The inheritance of bristles in *Setaria italica* has formed the subject of a previous article [Rangaswami Ayyangar, 1933]. Occasionally, in some races, some of these bristles bear spikelets at their tips. This occurrence together with a simple aspect of the inheritance of this peculiarity has formed the subject of a preliminary paper [Rangaswami Ayyangar, 1928]. This article presents the above and the summary of other experiences in the pursuit of the incidence and inheritance of this character of spikelet-tipped bristles.

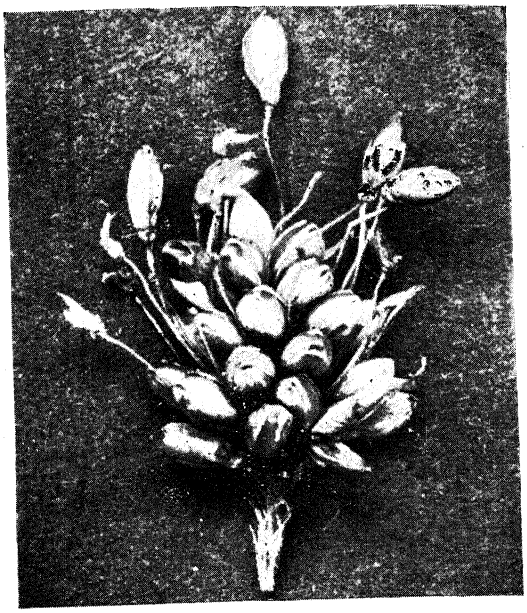
A spikelet in Setaria is a fascicle consisting usually of three units [Arber, 1931]. These may be in the nature of spikelets or bristles. The bristles are branched in *Setaria glauca*. This common origin, though differential expression, gives rise occasionally to evidences of affinity between the component parts of the fascicle. In *Setaria verticillata* the commonest expression of the fascicle is one spikelet and two bristles. In *Setaria italica* in the varieties examined, one spikelet and one prominent bristles is more common than one spikelet and two bristles. Instances have been met with in which some of the bristles were tipped with a spikelet. In *S. glauca* an extra spikelet rarely takes the place of a bristle brush. In *S. verticillata* bristles with rudimentary spikelets are occasionally met with. In the cultivated *Setaria italica* spikelets borne on bristles occur in greater frequency and are much better developed.

Nil. Stray. Full.

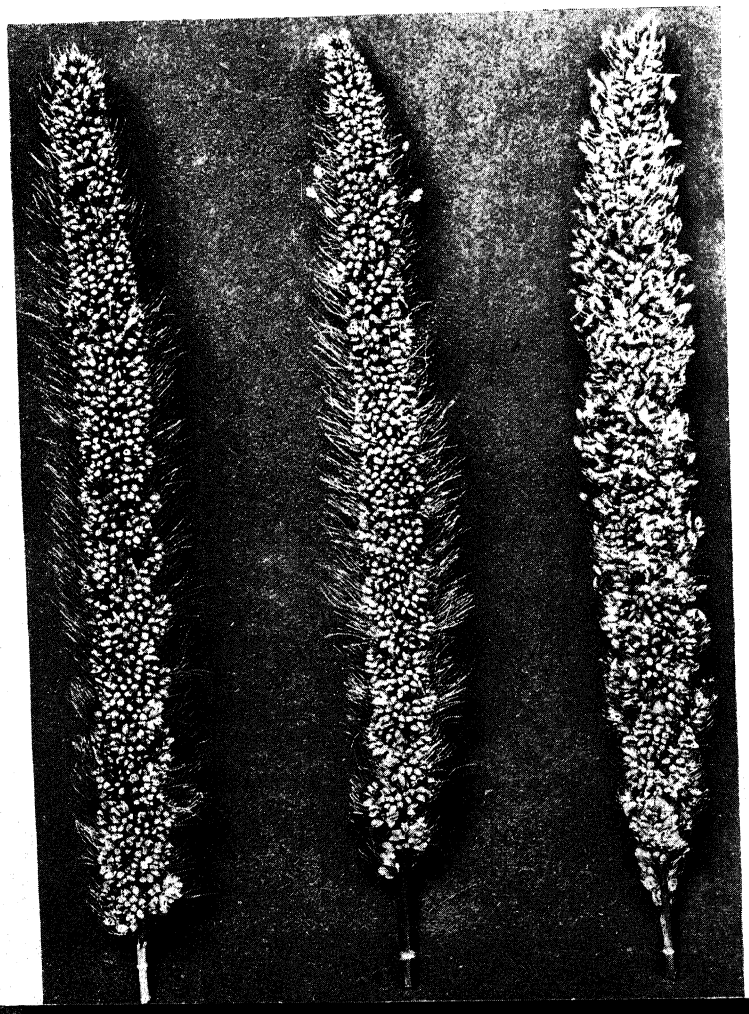
Spikelet-tipped bristles.



Free.



Spikelet-tipped.



The degree of development of these spikelets varies considerably. In some cases a small knob at the tip of the bristle is the only indication. In several cases only grainless glumes perch on the bristles. But in others a perfect grain is borne. In fact all stages of development from the rudiments of a spikelet to a well developed grain are met with.

The best developed among these pedicelled spikelets are slightly smaller and lighter than the normal grains of the head, but the germination percentage and energy of both kinds of grains is about the same.

As regards the degree of manifestation of this character two classes are recognisable—the 'stray' and the 'full'. The 'stray' is characterised not only by a sparseness of manifestation, but also by an indifferent development of these spikelets which results in their being mostly rudimentary or chaffy. The 'full' is a full both in expression and numbers. It is possible to find cases where about 80 per cent. of the bristles could be spikeletted and of these up to 35 per cent. could be well developed grains. When bristles bear no spikelets at their tips but are free, as in the ordinary Setarias, their condition has been designated 'nil', from the spikeletting point of view (Plate XXXIX).

The presence of a spikelet at the tip of a bristle results naturally in a shortening of the bristle, part of which it replaces. This shortening tendency of the spikelet is incompatible with the fullest expression of the bristle length so that the impact of the two, results in a necessary absence of any degree of full spikeletting in the long bristle, and the visible heterozygous condition of such an association is a sparseness of manifestation of spikelets at the tip of the bristles, which results in 'stray' spikeletting. In short, the 'long' bristle with 'stray' spikeletting represents the *modus vivendi* of spikeletting in the long bristle group. When long bristles are spikeletted, their incidence ranges from 4 to 16 per cent. of the total number of bristles.

The medium bristle is not intolerant of spikeletting and in a 'medium full' near 50 per cent. of the bristles bear spikelets and the incidence of rudimentary and unfilled grains is less. The tendency to reduce the combined length of the bristle and the spikelet at its tip is not much in evidence.

But the maximum manifestation of the character of spikeletting is met with in the 'short' group where, as many as 80 per cent. of the bristles may be spikeletted. When stray spikeletting occurs in the 'short' group about 10 per cent. of the bristles may bear good spikelets. In the short bristle the existence of the spikelet at the tip of the bristle tends to increase the combined bristle-spikelet length a bit more than the length of the free bristle. Spikeletting has not so far been noticeably met with in the 'dwarf' bristle.

The condition represented by a 'full' incidence of the spikelet has proved allelomorphic to its complete absence, *i.e.*, the normal condition designated 'nil'.

In the year 1925 a selection No. S. I. 20 b segregated and gave 96 'nil' spikeletted and 28 'full' spikeletted heads. The F_3 generation showed that the spikeletted bristle behaves as a simple monohybrid recessive. It is interesting that this functionally suggestive character revealing the origin and morphological nature of bristles should behave as a simple recessive dominated by strong inhibitory factors which in evolution are possibly responsible for dropping out the spikelets borne aloft and left the cultivated Setaria heads with mere bristles and their short stalked mass of spikelets.

The behaviour of the above family as well as of some others raised from crosses is presented in Table I.

TABLE I.

Segregating for 'nil' and 'full' spikeletting (3 : 1).

Season	Generation and family	Character of selection	Behaviour of spikeletting	
			'Nil'	'Full'
	S. I. 20 b (Natural cross of 1924)			
1925	F_2 S. I. 20 b	..	96	28
1926	F_3 S. I. 187, 188, 189, 192	'Nil'	Pure	..
	S. I. 190, 191	,,	346	119
	S. I. 193, 194, 195	'Full'	..	Pure
	From S. I. 190			
1927	F_4 S. I. 304, 305	'Nil'	Pure	..
	S. I. 306	,,	66	21
	S. I. 307	'Full'	..	Pure
	S. I. 308 (Natural cross of 1926)			
1927	F_2 S. I. 308	'Nil'	52	16
	S. I. Crosses XLI, XLII and XLIV			
1929	S. I. 195	..	..	♀
	S. I. 224	..	♂	..
1930	....	..	F_1	..
1931	F_2 S. I. 1905 to 1916	'Nil'	2225	656
	From S. I. 1905.			
1932	F_3 S. I. 2224, 2226, 2228, 2232, 2233	,,	Pure	..
	S. I. 2222, 2223, 2225, 2227, 2229, 2230, 2231.	,,	241	95
	From S. I. 1909.			
1932	F_3 S. I. 2236, 2237, 2239	'Nil'	Pure	..
	S. I. 2234, 2235, 2238, 2240, 2241	,,	54	20
	S. I. Crosses XLV and XLVI.			
1929	S. I. 195	..	..	♀
	S. I. 188	..	♂	..
1930	....	..	F_1	..
1931	F_2 S. I. 1917 to 1919	'Nil'	431	122

Further experiences have revealed a greater elaboration in the inheritance of this character. In a number of new families the simple dominance of the 'nil' condition is wanting and the heterozygote has a distinct expression intermediate between the ' nil ' and the ' full.' This is the 'stray' condition, and as indicated above a sparseness of manifestation of the spikelet-tipped bristles is the general feature in this group. This sparseness is confined to the tops of the earheads. There is naturally extreme variation in spikeletting in the ' stray ' group. It may partake of the nature of a few chaffy grains or even knobs with short bristles hardly visible above the grain surface of the head, or there may be such a manifestation of these pedicelled spikelets as possibly to run the risk of classification with the ' fulls '. But in every one of the cases ' strays ' spotted as natural crosses or picked from segregating families, have invariably segregated into the 1 : 2 : 1 ratio. There is thus no doubt as to the existence of an intermediate heterozygous group. This heterozygous group notwithstanding, the segregation is still of the monofactorial type and it is possible with some difficulty to classify the populations into ' nil ', ' stray ', and ' full ' and they show a close approximation to a 1 : 2 : 1 ratio. In further generations the ' nils ' and the ' fulls ' have bred true and all the ' strays ' segregated into the three groups. The behaviour of such families is detailed in Table II.

TABLE II.

Segregating for ' nil ', ' stray ' and ' full ' spikeletting (1 : 2 : 1).

Season	Generation and family	Character of selection	Behaviour of spikeletting		
			'Nil'	'Stray'	'Full'
	From S. I. 931 (Selection of 1928)				
1929	S. I. 1228, 1229, 1230	' Nil '	Pure	..	..
	S. I. 1231, 1232, 1233	' Stray '	191	356	195
	S. I. 1234, 1235	' Full '	..	...	Pure
	From S. I. 932				
	S. I. 1236, 1237, 1238	'Stray'	181	318	151
	Crosses I-IV.				
1928	S. I. 181				♀
	S. I. 224		♂		
1928	...			F_1	
1929	F_2 S. I. 1020-1029	'Stray'	580	1133	658
	From S. I. 1024				
1930	F_3 S. I. 1639-1644	,,	224	403	206

TABLE II—*contd.*

Season	Generation and family	Character of selection	BEHAVIOUR OF SPIKELETTING		
			'Nil'	'Stray'	'Full'
1930	F_3 *From S. I. 1025.*				
	S. I. 1646, 1653, 1654, 1656, 1658, 1663, 1664.	'Nil'	Pure	..	..
	S. I. 1645, 1647-1652, 1655, 1657, 1665-1668.	'Stray'	309	536	269
	S. I. 1659, 1660, 1661, 1662, 1669, 1670.	'Full'	..	..	Pure
	F_3 *From S. I. 1026.*				
	S. I. 1671, 1672, 1673, 1677-1680, 1682-1686, 1688, 1689.	'Stray'	322	673	333
	S. I. 1674, 1675, 1676, 1681, 1687, 1690.	'Full'	..	..	Pure
	F_3 *From S. I. 1027.*				
	S. I. 1697-1701, 1703-1712.	'Nil'	Pure	..	..
	S. I. 1691-1697, 1702.	'Stray'	202	353	172

The two sets of behaviour detailed above are distinct. The simple explanation of imperfect dominance is not satisfying enough. Further work is in progress to elucidate the inter-relationship between the two behaviours, as well as the broader setting of this character of spikeletting in its relation to its basic bristle.

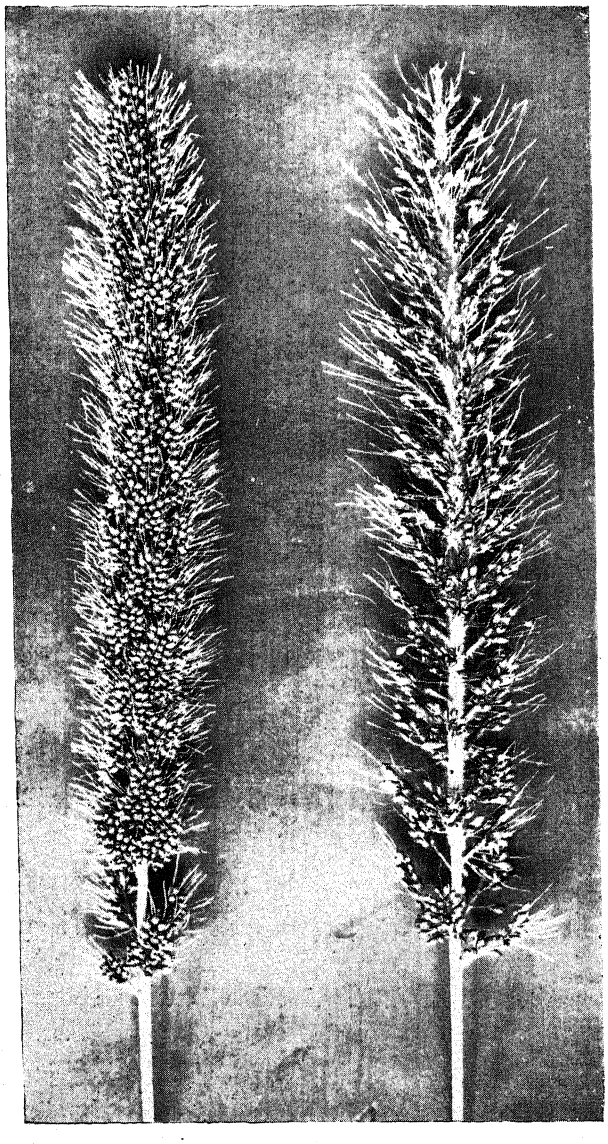
SUMMARY.

The bristles in Setaria occasionally bear an extra spikelet at their tips in some races. This is more common in the cultivated *Setaria italica* than in the wild *Setaria glauca* or *S. verticillata*, and behaves as a definite heritable character. The condition where most of the bristles are tipped with a spikelet is designated 'full' and it is allelomorphic to the 'nil' spikeletted bristle condition. The 'full' in some families has been found to be a simple monohybrid recessive to 'nil'.

In other families dominance is incomplete and an intermediate heterozygous class 'stray', where only a few bristles are spikeletted, is present, the three classes occurring in a 1 : 2 : 1 ratio.

The full spikeletted condition has been found to be incompatible with a long bristle.

The history of over 150 families representing both the types of inheritance is detailed.



Dense. Lax.

Ear-head density.

THE INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.), THE ITALIAN MILLET.

PART V. A TYPE OF LAX EARHEAD.

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(Received for publication on 12th October 1932)
(With Plate XL).

In the survey for seed material for work on this millet, some seeds were gathered from the Madakasira taluq of the Anantapur district. From the crop raised selection No. S. I. 2160 was made. This when sown was noticed segregating into the normal 'dense' earhead and a type of very 'lax' earhead (Plate XL). This F_2 generation gave 87 'dense' and 29 'lax' earheads. From this generation 12 'dense' and 3 'lax' heads were carried forward to a third generation. The behaviour of the families raised from these 15 earheads in the third generation is presented below :—

No.	Character of selection	F_3 BEHAVIOUR	
		'Dense'	'Lax'
S. I. 2254	'Lax'	..	Pure
S. I. 2255	,,	..	,,
S. I. 2256	,,	..	,,
S. I. 2246	'Dense'	Pure	..
S. I. 2249	,,	,,	..
S. I. 2242	,,	62	12
S. I. 2243	,,	64	12
S. I. 2244	,,	26	11
S. I. 2245	,,	18	5
S. I. 2247	,,	56	16
S. I. 2248	,,	122	48
S. I. 2250	,,	200	72
S. I. 2251	,,	132	41
S. I. 2252	,,	23	5
S. I. 2253	,,	100	30
Total		803	252

The segregation was clean and clear-cut, there being no gradations likely to lead to a confusion in the grouping. It is obvious that this differentiation is brought about by a single factor, provisionally designated A. The 'dense' heads had this factor A. The 'lax' heads lacked it.

This type of great laxity, so uncommon and so uneconomic but still so very definite, was pursued by an examination of the two groups of earheads. Heads from both groups were paired up and an analysis of 20 earheads from each of these shows that this type of laxness results in the combined effect of a lesser number of spikes, fewer number of grains per spike, and a chronic non-setting of the grains in the spikes. Details of this analysis are given below :—

Average of 20 heads	'Dense'	'Lax'
No. of spikes	121	105
No. of spikelets	2,660	1,100
Chaffy spikelets	22 per cent.	71 per cent.
No. of spikelet-tipped bristles	*Nil*	39
Bristle length	8·5 cm.,	10·5 cm.

It will be noticed that this single factor difference has meant such a sharp drop in the economic status of the 'lax' head. There were, and could be, no such 'lax-headed' varieties under cultivation. In a poor man's crop grown under dry-farming conditions, the elimination of the unfit is very quick. As such, the occurrence of this type of earhead can only be accounted for in terms of some kind of mutational origin. The primitiveness of the 'lax' earhead is evidenced by an increase in its bristle-length and by the presence of spikelets at the tips of some of the bristles. It was noticed that the 'lax' heads ripened a week later than the 'dense' ones.

It is remarkable that a single gene should be responsible for giving this sharp and unexpected glimpse into the history and evolution of the 'dense' earhead in this millet.

Summary.

A primitive type of 'lax' earhead characterised by fewer spikes, fewer spikelets, and chronic sterility, has suddenly occurred and behaved as a simple recessive to the normal economic 'dense' earhead. A factor A is set down to be responsible for this difference.

THE INHERITANCE OF CHARACTERS IN *SETARIA ITALICA* (BEAUV.), THE ITALIAN MILLET.

PART VI. ALBINISM.

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Albinism, the extreme manifestation of chlorophyll deficiency is commonly met with in cereals [Rangaswami Ayyangar, 1932]. In the cultivators' fields this lethal character has hardly any chance for survival, but it takes a long time for it to disappear. With a knowledge of its inheritance this undesirable character could be eliminated in a couple of years. The senior author has had his first opportunity of meeting with and working *in extenso* on this charactor in rice*. Coming to millets, in sorghum wide manifestations have occurred and have been pursued [Rangaswami Ayyangar and Sankara Ayyar, 1932]. In *Eleusine coracana* intensive work, including an artificial inducement of albinism has been possible [Rangaswami Ayyangar, *et al*, 1931]. This phenomenon has been met with in *Pennisetum typhoideum* [1932] and *Paspalum scrobiculatum* [1930].

This article records its occurrence in *Setaria italica*. In the year 1929 white seedlings were noticed to occur in a selection No. S. I. 517. There were 536 of these in a total population of 2240, leaving 1704 surviving greens. Twenty selections were carried forward and sown, and the behaviour of the third generation is recorded below :—

No.	F_3 BEHAVIOUR	
	Green	Albino
S. I. 1331, 1338, 1340, 1341, 1342, 1344, 1346, 1347	Pure	
S. I. 1332	632	215
S. I. 1333	685	226
S. I. 1334	574	199
S. I. 1335	801	276
S. I. 1336	580	214
S. I. 1337	667	220
S. I. 1339	684	233
S. I. 1343	376	123
S. I. 1345	739	236
S. I. 1348	641	243
S. I. 1349	321	106
S. I. 1350	488	160
Total	7,188	2,451

* Unpublished results submitted to Mr. F. R. Parnell.

It will be obvious that this is the usual mono-factorial type of albinism so common in cereals. A factor C_1 has been provisionally set down to be responsible for this character. When this character is met with, single plants (surviving greens) have to be selected and grown each separately. Those that do not throw white seedlings again, are pure for this C_1 factor and may be multiplied.

SUMMARY.

A simple segregation of mono-factorial type for green albino seedlings has been met with in *Setaria italica*. Factor C_1 is responsible for green seedlings, its absence resulting in albinos which do not live.

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STUDIES IN SETARIA ITALICA (BEAUV.), THE ITALIAN MILLET.

PART I.—ANTHESIS AND POLLINATION.

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(With Plate XLI and one text-figure.)

Introduction.

The study of the Italian millet has been in progress at the Millet Breeding Station of the Agricultural Research Institute, Coimbatore, from 1926. Observations on the anthesis of the crop were commenced in the year 1927 and continued in 1928. Some special bits of study were made in 1931. Altogether six sets of observations, three in the cold weather and three in the hot weather were made, comprising the study of thirty individual plants, during over 115 days and nights.

Anthesis is a very delicate process; and the observations recorded in one place need not necessarily apply in entirety to another, so much so that the drift of all cumulative work in this direction is to assay all environmental and physiological factors regulating this sensitive process.

Previous work.

There is not much previous literature on this subject. Knuth [1909] makes a passing reference to this millet in his classical work on pollination. Woodhouse and Ghosh [1911] make some general observations only. A short summary of the work of Hoshino and others [1926] in Korea, in which the periods of anthesis are given, is found in *Biological Abstracts*. In the absence of detailed information

it was thought desirable to undertake intensive work on this vital aspect in the life of this crop.

Material.—Typical plants from the following pure lines were chosen for observation :—

Year	Season	Pure line No.	No. of plants observed
1927	Hot	S. I.*74	2
		S. I. 77	1
	Cold	S. I. 46	4
1928	Hot	S. I. 77	2
		S. I. 284	2
	Cold	S. I. 46	2
		S. I. 63	2
1931	Hot	S. I. 566	2
		S. I. 1259	2
		S. I. 1717	3
	Cold	S. I. 310	2
		S. I. 312	2
		S. I. 1768	2
		S. I. 2053	2

* S. I. = *Setaria italica.*

Points studied.—The observations consisted of—

(1) A record of the sequence of anthesis, and

(2) Counts of the number of flowers opening at two hour intervals for the determination of :—

(*a*) The duration of flowering during a full day of 24 hours.

(*b*) The periods of maximum and minimum flowering.

(*c*) The number of days required to complete the flowering.

(*d*) The day in which the maximum number of flowers open, and

(*e*) The order of anthesis on the head.

Methods of observation.—Detailed observations on individual flowers, mature and about to open, were made, after clipping off, for the sake of convenience, other spikelets surrounding them.

The dehisced and shrivelled anthers with their slender filaments are easily blown off by wind and leave the opened flowers often unrecognisable. To avoid this possibility of confusion, opened flowers were then and there gently nipped off with a fine-pointed forceps.

The emergence of the inflorescence.

Unlike sorghum, the earhead of Italian millet is very long in proportion to its girth. This earhead shape dispenses with the well-marked 'boot' of sorghum. The first sign of flowering is a shortening of the distances between the top leaves. The 'flag', the erect terminal leaf blade, then comes out. This erectness of the flag continues till the head emerges, after which the angle it makes with the stalk of the earhead becomes a varietal characteristic. A week after the flag emerges, the earhead begins to push out of the opening at the top of the tubular flag-sheath. According to the variety, the head takes 10 to 20 days for complete emergence. There is a tendency to take a longer time in varieties with longer durations. The highest elongation occurs within the first five days. Often, the panicle begins to emerge even before the complete emergence, of the flag-sheath. The degree to which earheads are pushed out beyond the leaf-sheath varies in different varieties, the mass of heads being interspersed with leaf blades in varietes with poor emergence. During emergence the earheads themselves remain fairly constant in length, the increase in length being confined to their stalks.

The comparative rate of growth of single-stalked irrigated varieties, and tillering rain-fed ones, during the reproductive phase of their growth period was measured and shows that the period of rapid increase in height is (on an average) 18 days in rain-fed varietes and 14 days in irrigated ones. The rate of growth was 2·3 cm. per day in the former and 3·5 cm. in the latter.

Botany of the earhead.

Before proceeding to describe anthesis and its march on the earhead, a general description of the earhead, closely following Gammie [1911] is given below :—

Earhead.

Panicle.—Terminal, nodding, 5-35 cm. long and 1-4 cm. thick, usually compact, sometimes loose, cylindrical, ends often tapering, borne on a thin peduncle 2-25 cm. long. Made up of small rounded branches, the spikes, disposed in 3-5 spiral whorls along the rachis. Each spike containing up to 60 spikelets, the floral units. Spikes sparse at lower end and densely aggregated at upper half.

Rachis.—Greyish white, pilose,

Spikelets.—Sub-secund on the branches, glabrous, elliptic to obovate, 1-3 mm. long and 1 mm. broad, borne on thin, very short pedicels, slightly swollen at the junctions.

Bristles.—Each spikelet or 2 spikelets surrounded at base by a number of bristles which are thin, stiff, slightly flexuose, 3-15 mm. long, slightly flattened and set with minute upward-pointing barbs along the edges. Morphologically they represent barren floral branches.

Glumes.—Four varying in size.

Glume I.—$\frac{1}{3}$ of the spikelet, often even smaller, 3-nerved, nerves green.

Glume II.—$\frac{3}{4}$ of the spikelet, obovate, membranous, 5 full nerves and 2 partial ones on either side of the central nerve.

Glume III.—Equal in length to the spikelet, ovate-elliptic, 5 full and 2 partial nerves, membranous, paleate, sterile. *Palea* small membranous.

Glume IV.—Smaller than Glume III. Faintly rugose, increasing after seed setting and becoming bony, faintly 3-nerved, paleate, fertile, and containing one bi-sexual flower.

Palea.—Enclosed by Glume IV, membranous, edges incurved, two faint lateral nerves.

Stamens.—Three borne on slender filaments. Anthers 2-lobed, $\frac{3}{4}$ to 1 mm. long.

Ovary.—$\frac{1}{5}$ to $\frac{1}{4}$ of spikelet, hyaline.

Styles.—Two; distinct, divergent, hyaline, arising from a little below the apex of the ovary. Ends feathery. Styles 1 to $1\frac{1}{2}$ mm. Feathery portions $\frac{1}{4}$ to $\frac{1}{2}$ mm.

Lodicules.—Two; obcuneate, truncate, irregularly elliptic in cross section, situated on the side of the ovary nearest to Glume IV.

Seed.—Small, free, but tightly enclosed within the thickened glume and palea. Glume-side more convex than the palea-side which is rather flattened.

Occasional fertile third glume.

It is interesting to note that a race of the Italian millet has been met with at the Millet Breeding Station, in which up to 10 per cent. of the total spikelets bear a second grain inside the third glume—a fact reminiscent of the present abortive condition of what was once fertile.

Flowering.

First flowering.—The first flower to open can be looked out for, when about three-fourths of the head has emerged out of the sheath. This usually occurs a little below the apex of the head. Flowering proceeds from the top of the head downwards and similarly from the tip downwards in each of the panicle branches (Plate XLI.)

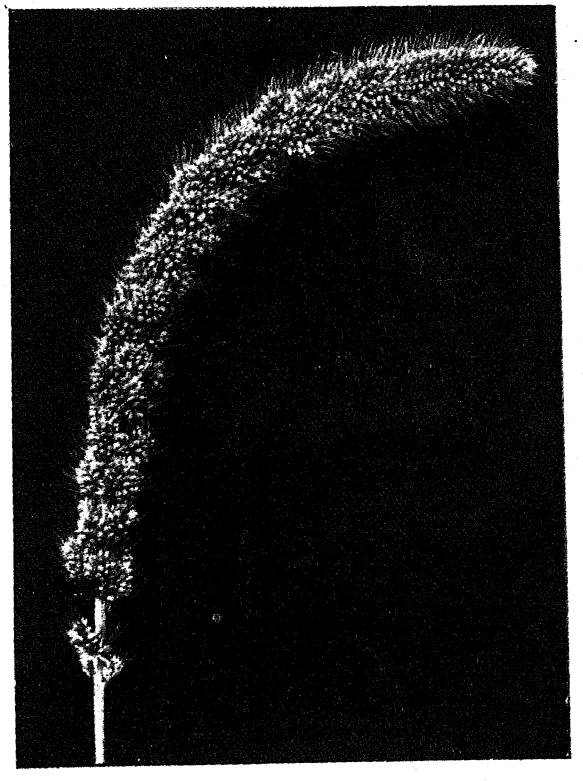


Fig. 1.—Earhead.

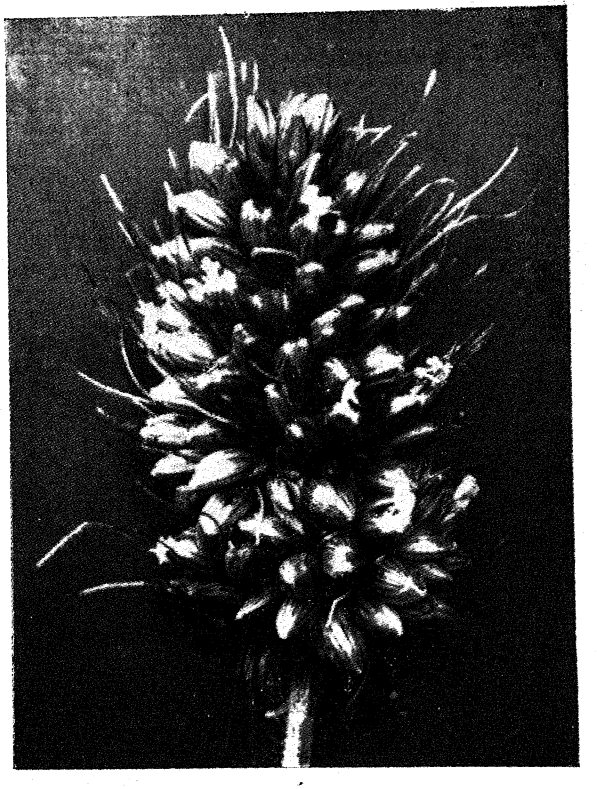
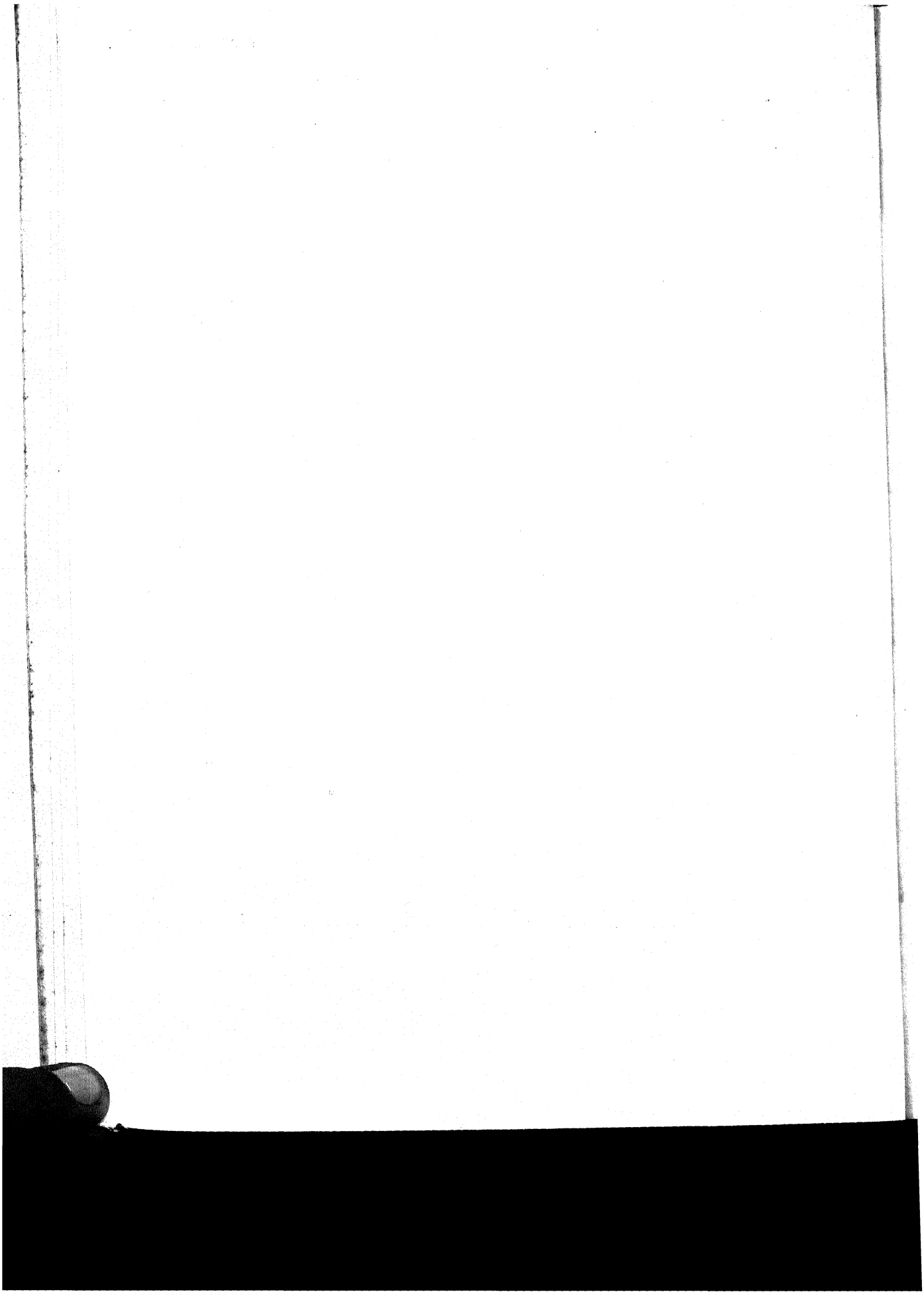


Fig. 2.—Spikes with open spikelets.

Setaria italica—Anthesis.



Opening of the flower.—Owing to the rapid swelling of the lodicules the glume and palea enclosing the ovary gape out, sometimes as wide as 40°. The pace of gaping is slow to begin with and increases in rapidity in the later stages. The gaping is so wide as to leave only the basal fourths of the floral envelopes overlapping.

About 8 minutes after the opening of the flower, the tips of stigmatic branches and one anther begin to protrude through the slit between the incurved edges of the palea. The stigmatic branches are the first to emerge, but are quickly followed and overtaken by the first anther, usually the one close to the lodicules. The first anther takes about 10 minutes to complete its emergence. The filaments which are about ½ mm. long prior to the opening of the flower now attain a length of 2 mm. and hold the anthers erect and aloft.

After the anther emerges it starts dehiscing by longitudinal slits from top to bottom, the process taking about 3 minutes. Prior to dehiscence there is a slight increase in the size of the anther and in the depth of its colour. This dehiscence liberates in clusters the pollen packed inside, which dusts the adjacent stigmas and parts of the surrounding spikelets. Emptied of pollen, the anther sacks flatten out, dry and turn brown. This proceess of emptying takes up to 10 minutes, increasing or decreasing according as the weather is chill or warm.

Five to ten minutes after the emergence of the first anther the other two are pushed out both at about the same time, and take their position one on each side of the first anther. Their growth and dehiscence are similar to those of the first anther, part of the pollen dusting the stigma of the same flower and the rest being broadcast. In the same flower the period during which the stigmatic feathers are open to be dusted with their own pollen is about 40 minutes, this period getting shortened with a quickening of all the other processes consequent on the advance of the day and its heat.

After pollination, the lodicules shrink and the glumes begin to close, after having been open for nearly an hour-and-a-half. The closing takes about half an hour. The rate of closing is rapid to begin with and slows down in the final stages. In the closed flower the stigmatic tips are clipped up, the feathers protruding beyond the glumes.

Self-pollination is thus the rule though extreme proximity to neighbouring flowers and the free nodding and contact with neighbouring heads does not preclude occasional cross-pollination.

The details of the processes in the anthesis of an individual flower are given below both for the hot weather and for the cold weather. The largest flush of flowering being before midnight, 12-midnight has been taken as the standard starting time.

Details of anthesis in a single typical flower of Setaria italica *in hot and cold weather.*

(Data, average from 82 flowers)

		Hot weather (May)	Cold weather (December)
Opening of the flower	Begins	12 midnight	12 midnight
	Complete	12-9 a. m.	12-18 a. m.
Emergence of the stigma	Begins	12-8 ,,	12-13 ,,
	Complete	12-17 ,,	12-28 ,,
Emergence of first anther	Begins	12-9 ,,	12-15 ,,
	Complete	12-18 ,,	12-33 ,,
Dehiscence ,, ,,	Begins	12-18 ,,	12-33 ,,
	Complete	12-20 ,,	12-37 ,,
Shrivelling ,, ,,	Begins	12-20 ,,	12-37 ,,
	Complete	12-24 ,,	12-45 ,,
Emergence of second anther	Begins	12-15 ,,	12-27 ,,
	Complete	12-21 ,,	12-42 ,,
Dehiscence ,, ,,	Begins	12-21 ,,	12-42 ,,
	Complete	12-23 ,,	12-46 ,,
Shrivelling ,, ,,	Begins	12-23 ,,	12-46 ,,
	Complete	12-27 ,,	12-54 ,,
Emergence of third anther	Begins	12-17 ,,	12-30 ,,
	Complete	12-23 ,,	12-45 ,,
Dehiscence ,, ,,	Begins	12-23 ,,	12-45 ,,
	Complete	12-25 ,,	12-49 ,,
Shrivelling ,, ,,	Begins	12-25 ,,	12-49 ,,
	Complete	12-29 ,,	12-57 ,,
Closing of the flower	Begins	12-40 ,,	1-0 ,,
	Complete	1-0 ,,	2-0 ,,

From the above representation the trend of the sequence of events is unmistakable. It will be seen that in the cold weather flowers may keep open for a period roughly twice that in the hot weather. This is brought about by a slight lengthening in the time taken over every item of the anthesis, there being a marked rise in the time taken for the glumes to close.

Within a day the extent of remaining open naturally varies within wide limits, it being longer in the cool late hours of the night and shorter after sunrise.

Period of flowering.

The time taken for an earhead to complete its flowering, will vary with its size —the bigger the longer—but from 10 to 15 days may be taken as the range. The average number of flowers that open per day, both in the cold weather (rain-fed) and in the hot weather (irrigated) based on readings from 14 earheads in each of the seasons is given below, and points to the rate of opening, showing a good rise and a gradual decline during the period, the rise and fall being a bit sharper in the hot weather.

Number or flowers opening per day.

(Average of 14 heads)

Day	Hot weather (irrigated)	Cold weather (rain-fed)
1st	49	26
2nd	320	143
3rd	478	232
4th	513	278
5th	471	287
6th	329	262
7th	234	218
8th	180	146
9th	165	110
10th	132	68
11th	83	46
12th	27	25
13th	7	12
14th	2	7
15th	1	2

Range of daily flowering.

The most interesting feature in the anthesis of this millet is in the periodicity of its flowering during a whole day, including the night. The following table gives the distribution of flowering at two hour intervals during a whole day, in the hot weather and in the cold weather.

Distribution of flowering during a day.

(Average of 14 heads)

Time of the day	Hot weather		Cold weather	
	Average temperature	No. of flowers opening	Average temperature	No. of flowers opening
12-2 p.m.	98	3	81	..
2-4 „	96	3	81	..
4-6 „	91	6	78	..
6-8 „	86	38	71	1
8-10 „	83	309	68	134
10-12 midnight	81	751	66	580
12-2 a.m.	79	365	65	348
2-4 „	77	279	64	178
4-6 „	76	364	63	182
6-8 „	82	672	66	290
8-10 „	88	168	74	159
10-12 noon	94	23	79	4

It will be noticed from the above table that whereas stray flowering might occur between 10 a.m. and 8 p.m. the main period is between 8 p.m. and 10 a.m. There are two maxima of flowering during this period, one between 10 p.m. and 12 midnight and the other between 6 a.m. and 8 a.m. The second flush in the hot weather approaches the first, while in the cold weather it is only half its intensity. This difference is very likely due to the fact that after the cold of the early hours of the morning in the hot weather there is a sharper rise in temperature between 6 a.m. and 8 a.m. than in the cold weather.

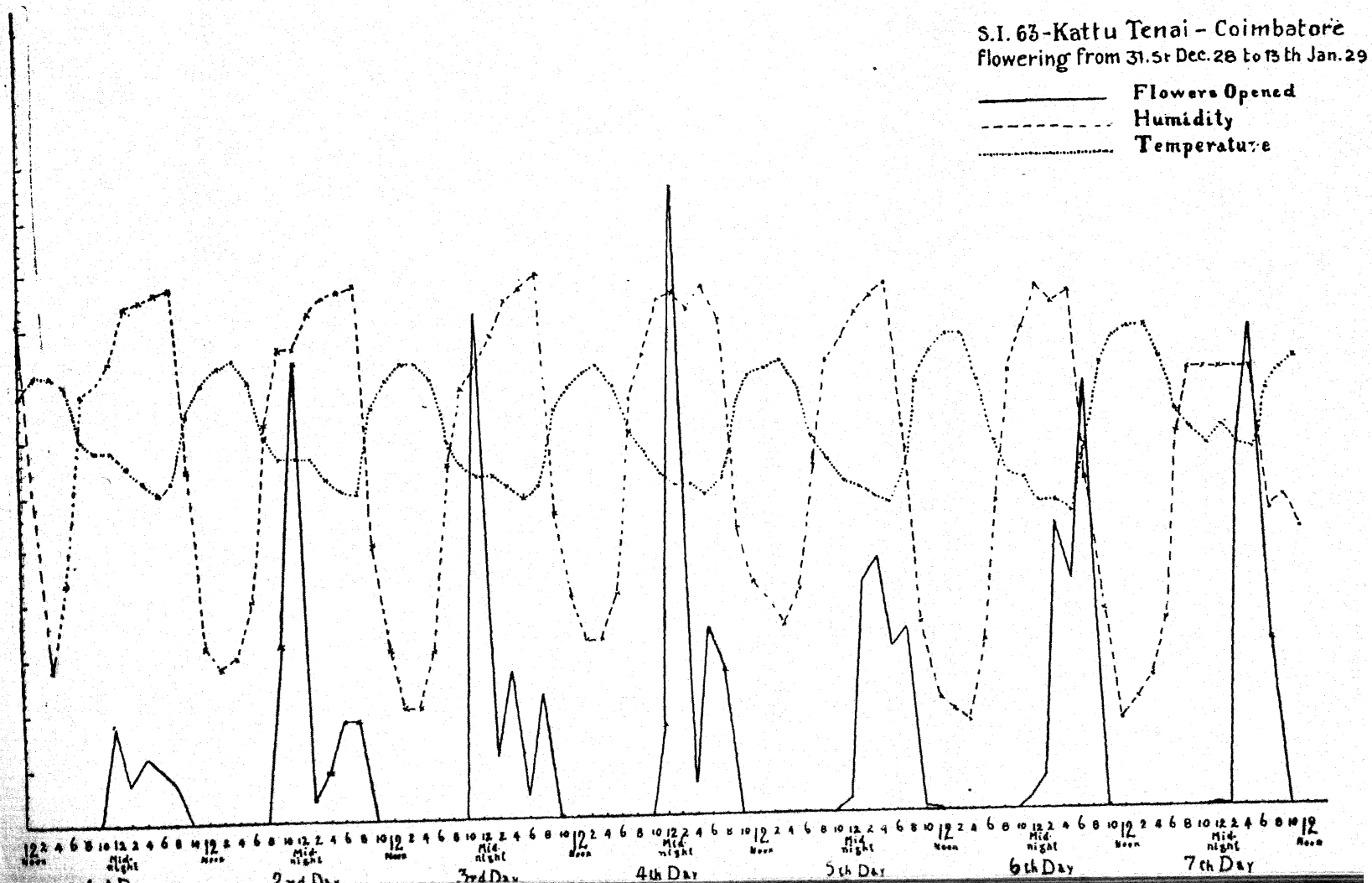
Anthesis, humidity and temperature.

The typical anthesis in one earhead along with the corresponding humidity and temperature records are charted out in the graph (Fig. 1). This shows that flowering does not occur in that part of the day when temperature is high and the humidity low and that it is confined to the period of low temperature and high humidity.

SETARIA ITALICA **ANTHESIS, HUMIDITY & TEMPERATURE**

SCALE

1 Horizontal division = 2 Hours
1 Vertical division = 3 Flowers opened,
2 Degrees of temperature, and
2 Per cent. humidity



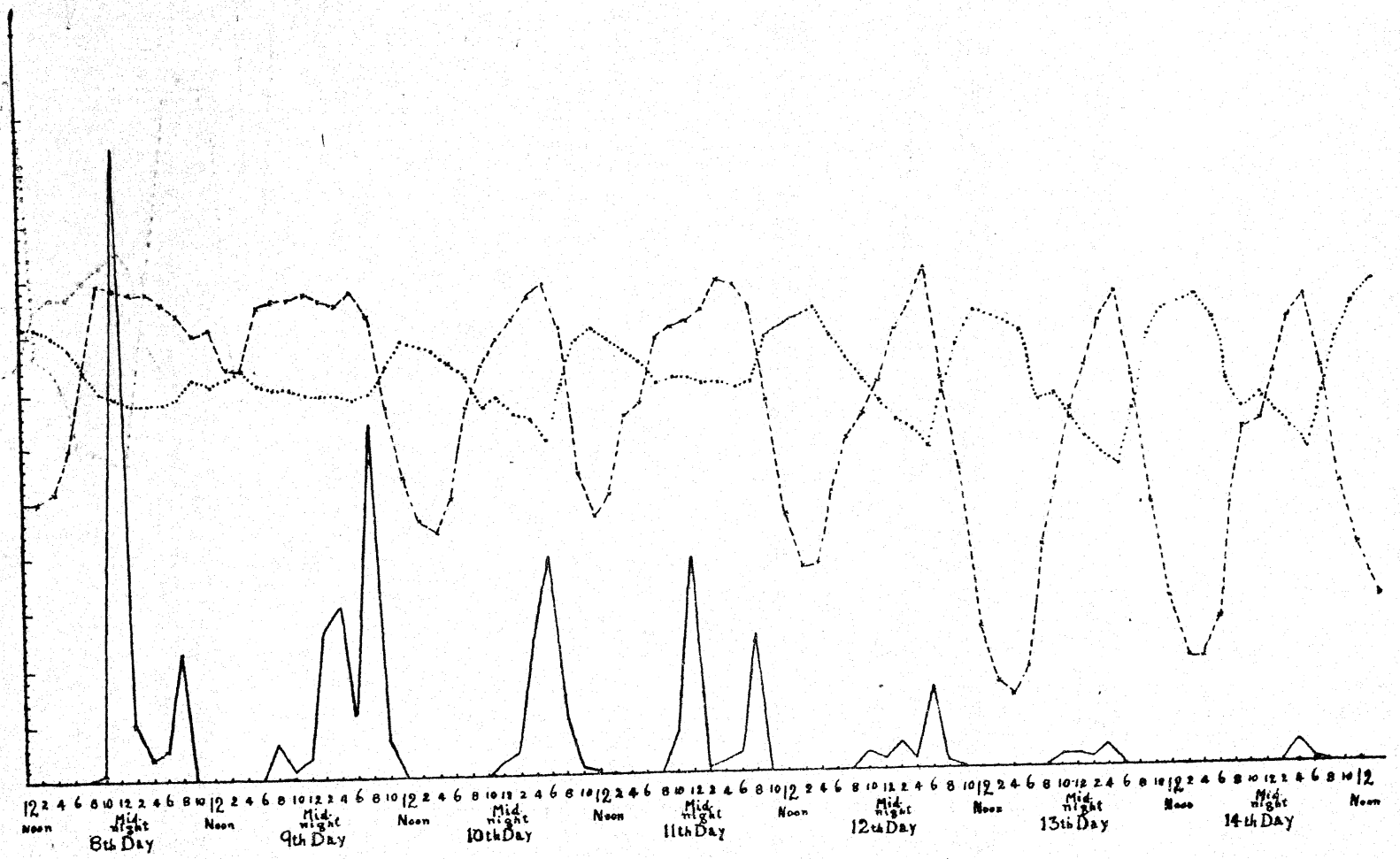

12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12 2 4 6 8 10 12
Noon
Mid night
Noon
Mid night
Noon
Mid night
Noon
Mid night
Noon
Mid night
Noon
Mid night
Noon
Mid night
Noon
8th Day
9th Day
10th Day
11th Day
12th Day
13th Day
14th Day

Fig. 1.

ANTHESIS INDEPENDENT OF VARIETAL CHARACTERS.

The bristles give a fox-tail look to this millet and are its prominent feature. To determine whether these inevitable appendages had any differential influence according to the degree of their manifestation, observations were made in the cold weather of 1931 on two sister families S. I. 310 and S. I. 312, one with long bristles and the other with short-bristles. The results showed that neither in the time taken for the head to complete flowering nor in the rate at which this was accomplished, nor in the two hourly counts made day and night, had the bristles exerted any influence.

The bristles are morphologically modified floral branches. Certain races are met with in which stray spikelets are borne at the tips of some of these bristles. Earheads with these extra spikelets are jagged in outline and look fuller. Naturally they have a larger number of flowers. This radical difference between normal and extra-spikeletted heads led to extending the floral observations to this type of earhead also. Both the types of earheads were observed and no deviations from the usual routine of floral behaviour were noticeable in all the details of anthesis.

Similar observations on paired earheads were made on white and brownish orange-anthered plants as also on buff and red-grained varieties. None of these showed any differences from the normal behaviour. It will thus be seen that varietal differences exert no noticeable effect on the general run of floral conduct in this millet.

SUMMARY.

The anthesis in *Seteria italica* has been studied. An earhead may take 10-15 days to complete its flowering, depending upon the size. The flowers open through the period 8 p.m. to 10 a.m., though in summer stray flowers may open between 10 a.m. and 8 p.m. There is a lull in the hot part of the day corresponding to rise in temperature and a fall in humidity. With the increase in humidity and the fall in temperature flowering commences and becomes intense towards midnight after which there is a slow fall and a second flush soon after sunrise. The floral arrangement favours self-pollination. Stray crossing might occur. Bristle variations, spikelet-tipped bristles, anther and grain colour differences, do not affect the general tenor of anthesis.

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ABSTRACTS

The basis for *Sclerospora sorghi* as a species. W. H. WESTON, Jr., and B. N. UPPAL. *Phytopathology* **22**, 573-586, 1932.

The history of the fungus causing the downy mildew of sorghum, which was first recognised by Kulkarni in 1913 as a variety, *Andropogonis sorghi*, of *Sclerospora graminicola* is reviewed.

Significant structural details of the conidial phase of this fungus on sorghum are compared with those of typical *Scl. graminicola* on *Pennisetum* and *Setaria*. The fungus on sorghum differs distinctively in its absolute lack of a dehiscence, papilla in the apical wall of the conidia and their consequent germination by hyphæ, and in the definite basal cell, extensive branch system, and consequent arrangement of the conidia in a hemispherical plane on the longer sterigmata of the conidiophores. On these features the fungus is separated from *Scl. graminicola* the specific rank as *Scl. sorghi* (Kulk.) Weston and Uppal.

The oogonial phase resembles that of *Scl. graminicola* in general characteristics, but cross-inoculation experiments, using oospore material as inoculum, show that *Scl. sorghi* will not inoculate Setaria and Pennisetum, nor will *Scl. graminicola* from Pennisetum or Setaria inoculate sorghum, in spite of successful inoculations from each of these hosts to such remotely related Gramineæ as *Euchlæna mexicana*. The results of these inoculations which offer corroborative evidence for recognizing the fungus as a distinct species, are described and tabulated. (B. N. U.)

Two new hosts of the downy mildew of sorghum in Bombay. B. N. UPPAL and M. K. DESAI. *Phytopathology* **22**, 587-594, 1932.

The results (which are discussed and tabulated) of the writers' investigations at Poona show that the downy mildew of maize, which is found occurring naturally on this host in the Bombay Presidency, is caused by *Sclerospora graminicola* var. *Andropogonis sorghi*. In the conidial stage it is not possible to distinguish between the mildews on sorghum and maize. The sexual stage is prominent on sorghum, resulting in the characteristic shredding of leaves, but it is completely suppressed in maize.

Maize and teosinte were infected with the oospores of *Scl. graminicola* var. *Andropogonis sorghi* under controlled conditions.

A comparative morphological study of the conidial phase of the mildew as it occurs naturally on maize and sorghum in Bombay was made. In general appearance the conidiophores are alike, and each is provided with a basal cell. The biometrical measurements of the conidia of the mildews on maize and sorghum have shown that the differences in length and width of conidia are not statistically significant, thus indicating that the two fungi are identical. (B. N. U.).

A study of the oil from the niger seed (*Guizotia abissinica*). D. L. SAHASRABUDDHE and N. P. KALE. *Journal of the University of Bombay* **1**, Part II.

This study was undertaken in order to determine the chemical nature of the oil and to get an idea of the various uses to which the oil can be put. The oil gives the following constants :—

Sp. gr. 0.9157 at 28·6°C.	Refractive index at 40°C. (N_d) 1·4662.
M. pt.-7·5 to 8·5°C.	Free acidity 3·8 to 4 mgms. KOH per gram of oil.
Acetyl value (Lewkowitch and Andre) 24·1.	Saponification value 194·6.

Richert-Meissl number 0·85 — Iodine value 126·4 per cent. (Wij's method).

Insoluble fatty acids 94·3 per cent. — Bromine absorption 79·8 per cent. (Wij's method).

The insoluble fatty acids give the following composition:—

85·4 per cent. unsaturated acids made up of	31·06 per cent. oleic.	
	54·34 per cent. linolic.	
14·6 per cent. saturated acid „ „	0·35 per cent. lauric and myristic.	
	8·41 „ palmitic.	
	4·89 „ stearic.	
	0·48 „ arachidic and lignoceric.	
100·00		100·00

On bromination the oil gives 3 to 4 per cent. of a tri-linolein-bromide melting at 76·5°C. and 20 to 25 per cent. of di-lino-olein-bromide melting at 54 to 56°C.

Completely saturated glyceride is not present in the oil.

The raw oil when kept for some time becomes rancid, due to hydrolysis. It also thickens indicating polymerisation. Peroxide bodies giving the starch iodide reaction are formed to a small extent. This polymerisation seems to be due to enzymic action which is stopped in the absence of air. If the oil is heated to 110°C. to 120°C. and is kept out of contact with air it remains unchanged for a long time.

It takes up sulphur chloride at ordinary temperatures so energetically that the reacting mass has to be cooled to avoid charring. The product of reaction is a white, elastic rubber-like substance.

The oil on hydrogenation gives a white solid fat. The hydrogenation proceeds normally, no iso-oleic acid being formed. (D. L. S.)

REVIEW

The Methods of Statistics. By L. H. C. TIPPETT. (London: Williams and Norgate Ltd., 1932). 15*s*. net.

This book will be welcomed by most biologists who have to deal with observational data and to test their mathematical reliability. In recent years there have appeared numerous books on this science, but they deal with methods and concepts formulated by the author himself and hence they present the subject in a more restricted sense. The present author has, however, dealt with the subject in a general manner and has attempted to present a single system of statistics so that the reader may obtain a good working knowledge and understanding of the different methods available. After dealing with frequency distribution, the author has explained in a very lucid manner "the theory of probability" and its bearing on statistical science. He has then dealt with the theory of 'Random Sampling' with which is associated the probable and standard errors, all of which the reader needs to understand before he can follow the subsequent chapters on 'Goodness of Fit,' the 'Analysis of Variance' and 'Correlation'. The chapter on 'Goodness of Fit' and contingency tables explains clearly the function and properties of Pearson's x^2 with various degrees of freedom. The methods so far mentioned are limited to large samples, but it is not always possible for biologists to obtain such samples and especially in agricultural experiments, it is constantly found necessary to interpret results obtained from small samples. The author has, therefore, described methods by which the data from small samples can also be statistically interpreted.

The chapter on 'Analysis of Variance' explains in simple terms the methods employed for the analysis and the computation of variances from grouped data. The underlying theme of this and later chapters is Fisher's idea of the analysis of variance. The additive nature of those variances which are subject to the operation of several independent causes and their non-additive nature in cases where the observations are not independent and an association between the variates, has been treated in clear and simple language and made easy to understand by means of examples. After dealing with the variance in which one of the variates is qualitative and is associated with a quantitative variate, the reader is introduced to associations in which both the variates are quantitative, when they are known as correlations. The procedure adopted in presenting correlation as a special case of variance is unique and brings out the unity of idea more clearly than by any other method of presentation. The author has taken pains to explain the relation that exists between correlation and regression co-efficients and, with the help of

tables and diagrams, the frequency surfaces for different values of correlation co-efficients have been illustrated so as to enable the reader to understand their distribution. The sampling distribution of the correlation co-efficients forms the subject matter of next chapter. The methods for dealing with the distribution of correlation co-efficients on account of the errors of random sampling and tests of its significance are explained here. While dealing with correlation co-efficients, the author has also explained the regression line and regression co-efficient, but the non-linear regression has been presented in a separate chapter, as in such cases the correlation co-efficient is not a reliable measure of association but another constant, the correlation ratio, has to be used. This is calculated from array means in large samples and from fitted curves in small ones. As a side issue of non-linear regression, intra-class correlations and correlation of ranks is dealt with in this chapter. Multiple and partial correlations and regression are dealt in a separate chapter with which the book ends.

The most important chapter from the point of view of an agriculturist is the one dealing with 'Principles of Experimental Arrangement'. After showing how the heterogeneity of variability has an important effect on the theory of errors, the principles involved in the experimental arrangement of plots are explained. In dealing with the arrangement of plot for field trials in agricultural experiments the various methods such as "Chessboard", "Latin Square", etc., are discussed and the statistical principles involved in each are explained and finally the author emphasises the importance of technical considerations, such as the effect of weather, provision of borders, etc.

The book at first gives the impression that the grasping of its subject-matter calls for a good knowledge of higher mathematics as it is full of mathematical formulæ and equations. Actually even those who have to omit the mathematics entirely should be able to arrive at an appreciation of the arguments, for the statistical principles involved have been made clear and easy to understand by the worked examples.

The book is a valuable addition to the existing literature on the subject and should prove of great help to biologists and statisticians alike.

The whole subject has been presented in a delightful style and well illustrated with tables and figures for which the author deserves congratulation. (M. A.)

THE MAYNARD-GANGA RAM PRIZE.

In 1925 the late Sir Ganga Ram, Kt., C.I.E., M.V.O., R.B., Lahore, with that generosity for which he was so well known, handed over to the Punjab Government a sum of Rs. 25,000 for the endowment of a prize of the value of Rs. 3,000 to be called the Maynard-Ganga Ram Prize and to be awarded every three years, for a discovery, or an invention, or a new practical method which will tend to increase agricultural production in the Punjab on a paying basis. The competition is open to all throughout the world. Government servants are also eligible to compete for it.

Applications for the next award were invited by the 31st December, 1932. The response was, however, poor and it has been decided by the Managing Committee of the above-mentioned prize that the award should be postponed for another year and that further applications should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1933.

* * *

Applications are invited for "The Maynard-Ganga Ram Prize" of the value Rs. 3,000 which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not eligible for competition and applicants should prove that some part of their discovery, invention, etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All applications in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1933.

ORIGINAL ARTICLES

BLOOMING AND ANTHESIS IN KOLAMBA RICE.

BY

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I. INTRODUCTION.

In improvement of crops by genetical methods the time ultimately arrives when hybridization is resorted to. A close familiarity with the inflorescence, mode of flowering and various phases of anthesis is useful to develop technique of crossing and to increase chances of success.

The blooming in rice has been observed by many workers in various climes. From these observations two facts stand out conspicuously, namely, that the

varietal and climatic variations largely influence blooming and associated phases in rice. These two factors make it necessary to study blooming process and allied phases wherever rice improvement is in progress.

Observations were accordingly made on blooming and anthesis in the Kolamba strains during the season of 1931, at the Rice Breeding Station, Karjat, District Kolaba, Bombay Presidency. The data obtained during the season forms the subject of this paper.

II. Previous observations.

Ramiah [1927], working at Coimbatore, observed that cultivated rice usually blooms between 9 a.m. and 12 noon and the maximum blooming is at 10 a.m. He also found that the varieties began to bloom as the temperature reached 77°F. or above and that during the six-day period the hour of blooming varied from 10-30 a.m. to 12-20 p.m., depending upon the time of day when temperature reached the 77°F. mark or above.

The observations of Rao [1926], in the Tanjore district, indicate that rice blooms from 5-30 a.m. to 4-40 p.m., but most of the blooming occurs between 9-45 and 10-30 a.m. The temperature ranged between 79° to 88°F. when the florets opened. The spikelets required from $1\frac{1}{2}$ to 3 minutes to open and the closing range was from twenty-seven to fifty-four minutes. Rao also observed that anthers dehisce just before or at the moment the flower begins to open.

Bhide [1925], working previously at Karjat, mentions that the flowers in the Kolamba rice begin to open at 10 a.m. and continue until 12 noon. He opines that the most active period is from 10-30 to 11 a.m. The early varieties begin to open as the temperature reaches 79° to 84°F., whereas the late ones commence flowering between 85° and 90°F.

Bhide and Bhalerao [1927] state that florets take seven to eight minutes to open completely and remain so far about fourty-five to fifty minutes and "then close up within about five minutes or so". The entire period from opening to closing requires from fifty-five to sixty minutes.

Hector [1913], working in Lower Bengal, reports that in early rice varieties flowering begins between 7 and 8 a.m., whereas late ripening varieties begin to open at 10 a.m. and continue until 12 noon. He also observed that the florets remain open thirty minutes in early varieties which bloom during the warmer and moist months of May and June, whereas in late varieties the duration is twice as long due to colder and drier climatic conditions obtaining during the months of October and November. Hector also observed that as a rule dehiscence of anthers and pollination take place simultaneously.

Thompstone [1915], in Upper Burma, reports that blooming usually takes place between 7 and 10 a.m., and is most active between 8 and 9 a.m. He found dewy mornings to be favourable and as the dew begins to disappear the flowers open rapidly, but as the day begins to get bright, warm and dry, the flowers cease opening and those already opened close again. This observer also states that the anthers burst as the flower opens.

Torres [1923] and Rodrigo [1925], observed that the blooming commences between 9 and 11-30 a.m. in the Philippine Islands. The latter reports that the flowers occasionally opened between 6 and 9 a.m., and between 11-30 a.m. and 12-30 p.m. On an average the flowers remained open from forty-five to sixty minutes, and a panicle required from five to ten days to finish blooming. Rodrigo states that in the majority of cases dehiscence and pollination occurred simultaneously as the flower opened.

Laude and Stansel [1927], from Texas, U. S. A., mention that the blooming in rice continues from 8 a.m. to 4 p.m., the peak occurring from 11 a.m. to 12 noon. The majority of the panicles finished flowering in six or seven days and most of the flowers bloomed on the second and third day after the commencement of blooming.

The American observers also report that pollination takes place just before or at the time the spikelet begins to open.

III. Material and methods.

Observations on various phases of blooming in rice were made during the season of 1931 at the Rice Breeding Station, Karjat, District Kolaba. The material consisted of five established strains, obtained by pure-line selection from the Kolamba variety of rice.

All the strains were sown in the beginning of the monsoon on June 13th, 1931, and transplanted in the fields during the second week of July, 1931. Each hill, eight inches apart, received only one seedling in rows spaced one and a half foot. The early strains, K79 and K184, were grown side by side on a high-level plot. The mid-late K153 was on a plot of medium level and the two late strains were transplanted side by side on a low-level plot. From each strain twenty main panicles were taken for observation. Records on the opening of flowers were taken at intervals of one hour, from 7 a.m. to 5 p.m. Temperature figures were obtained from a thermograph.* To preclude recounting an opened floret all the spikelets that had bloomed during the previous hour were clipped off.

The order of blooming in the rice plant was determined by observing blooming in main (first) panicles of each strain. At the same time as many individual

*Temperature data kindly supplied by Mr. S. K. Sannabadati, B. Ag., at the Rice Research Station, Karjat.

florets as was possible were watched for (1) time required to open, (2) time taken to remain *completely* open, and (3) time required to close completely.

IV. Observations and results.

1. Inflorescence of rice.

The inflorescence of rice is a branched terminal panicle of perfect flowers. The branches are composed of groups of three or more spikelets. The flower consists of an ovary with a forked feathery stigma and six stamens enclosed by lemma and palea. At the base of the ovary there are two fleshy lodicules. Outside the lemma and palea are two short outer or " empty " glumes.*

2. Panicle emergence.

The rice panicle emerges four or five days after the flag is completely out. The emergence takes place either during day or night. The day on which the panicle tip is observed to emerge is reckoned as the flowering day. On the second and third day most of the panicle is out and completely on the fourth day.

3. Rice panicle.

The primary and the secondary branches of the rice panicle are arranged alternately along the main axis. In some strains, notably K184, K153 and K226 the secondary branches are also branched. The number of sub-branches on a primary branch varies according to the strain, and whether the earhead is from the main or secondary culms. As a rule, the main panicle is larger in every respect than the other earheads of the same plant. The branches on the main panicle vary from nine to twelve and sometimes more.

The number of secondary branches on a primary branch also varies. The primary branches in the middle of the panicle are longer and generally possess a larger number, usually seven to eight, of secondary branches. In one case a primary branch had nine secondary branches. Generally, the basal primary branch has the fewest—three to four—sub-branches.

4. Order of blooming in the rice panicle.

The rice panicle usually commences to bloom on the morning of the following day after emergence. The flowering proceeds from top downwards. As an example a case of a typical panicle from K79 may be cited. The panicle emerged on the 7th September, 1931. The next day it commenced blooming with the first three primary branches. The first (terminal) branch finished blooming on the 9th

*In some rice varieties the outer glumes are as long or nearly so as the inner glumes.

September. On the same day as many as nine branches were still flowering and all the twelve on the 10th. The second, third and the fourth branches completed blooming on the 10th, the fifth and the ninth on the 11th and the rest on the 12th September, 1931.

5. *Arrangement and order of blooming of spikelets.*

The arrangement of the spikelets on sub-branches is also alternate. Indeed, the florets are so arranged that they follow a fairly regular course in blooming. Each group of spikelets behaves as a unit as far as flowering is concerned and is independent of any other in the same primary branch.

In the table below data are presented on the order of blooming in a panicle. It will be noted that the terminal floret is first to bloom in most cases. Out of the sixty-one sub-branches, fifty-eight had their terminal floret bloom first. In only three branches the lowermost floret opened first. In the majority of the cases the last or the basal floret blooms second; not infrequently, however, other florets follow the first flower in opening.

TABLE I.

Order of blooming of spikelets in a rice panicle.

Branch		Floret		Branch		Floret	
Primary	Secondary	First to open	Second to open	Primary	Secondary	First to open	Second to open
I	1st*	Basal	Terminal	III	1st	Terminal	Basal
	2nd	Terminal	2nd		2nd	,,	,,
	3rd	,,	Basal		3rd	,,	,,
	4th	,,	,,		4th	,,	,,
					5th	,,	,,
II	1st	Basal	4th	IV			
	2nd	Terminal	Basal		1st	,,	,,
	3rd	,,	,,		2nd	,,	2nd
	4th	,,	,,		3rd	,,	,,
	5th	,,	,,		4th	,,	,,
					5th	,,	Basal
					6th	,,	,,

TABLE I—*contd.*

Order of blooming of spikelets in a rice panicle—contd.

Branch		Floret		Branch		Floret	
Primary	Secondary	First to open	Second to open	Primary	Secondary	First to open	Second to open
V	1st	Terminal	Basal	VIII	1st	Terminal	Basal
	2nd	,,	,,		2nd	,,	,,
	3rd	,,	2nd		3rd	,,	,,
	4th	,,	Basal		4th	,,	3rd
	5th	,,	2nd		5th	,,	Basal
	6th	,,	,,				
	7th	,,	Basal	IX	1st	,,	,,
					2nd	,,	,,
					3rd	,,	,,
VI	1st	,,	,,		4th	,,	,,
	2nd	,,	,,				
	3rd	,,	,,	X	1st	,,	,,
	4th	,,	2nd		2nd	,,	,,
	5th	,,	Basal		3rd	,,	,,
	6th	,,	,,		4th	,,	,,
	7th	Basal	Terminal		5th	,,	,,
				XI	1st	,,	,,
VII	1st	Terminal	5th		2nd	,,	,,
	2nd	,,	Basal		3rd	,,	,,
	3rd	,,	,,		4th	,,	2nd
	4th	,,	,,				
	5th	,,	,,	XII	1st	,,	4th
	6th	,,	,,		2nd	,,	Basal
					3rd	,,	,,

*The terminal group of spikelets of a primary branch is designated as the 1st secondary branch.

After the terminal and the basal flowers have bloomed, the spikelets in a sub-branch flower alternately from below upwards in the majority of cases. The order is reversed in a considerable number. In a few cases, after the first and the last flowers have bloomed, flowering may be irregular.

A few cases of cleistogamic spikelets were observed.

6. *Daily blooming in a rice panicle.*

Observations on daily blooming throughout the entire flowering period were made on single panicles of each strain.

In the table below data are recorded on blooming of a main panicle from the Early Strain K79. The panicle commenced flowering on the morning of 8th September 1931. No flowers were observed to bloom before 10 a.m. and after 2 p.m. The modal flowering was between 11 a.m. and 12 noon. The maximum flowering occur-

red on the third day after the commencement, the panicle completed blooming in five days.

TABLE II.

Hourly bloom count on a main panicle of the early Kolamba strain K79.

Date	Hour							Total florets	Percentage
	7-8 a.m.	8-9 a.m.	9-10 a.m.	10-11 a.m.	11 a.m.-12 noon	12 noon-1 p.m.	1-2 p.m.		
8th Sept. 31	..	..	..	6	10	..	..	16	6·42
9th „ 31	..	..	..	6	52	4	..	62	24·90
10th „ 31	..	..	..	19	76	..	..	95	38·15
11th „ 31	..	..	..	1	39	2	1	43	17·27
12th „ 31	..	..	..	..	29	4	..	33	13·25
Total florets	..	..	..	32	206	10	1	249	..
Percentage	..	..	..	12·85	82·73	4·02	0·40		

7. Daily blooming in strain populations.

As stated above twenty panicles of each strain were taken collectively to observe the range and the amount of blooming at various hours. As an illustration, only the data on K42 are given in Table III.

In the early strains, K79 and K184, no appreciable differences were noted. Both strains show the maximum flush—about 50 per cent.—between 11 a.m. and 12 noon. In K79 nearly 28 per cent. of the blooming occurs *after* 12 noon, whereas in K184 nearly 23 per cent. of the flowers open *before* the hour of maximum flush.

Unlike other strains, K153 showed a longer range of maximum flush, about 88 per cent. of the blooming occurring between 10 a.m. and 12 noon.

The late strains showed conspicuous differences in their amount of blooming at various hours. It will be seen from the table below that K42 starts blooming vigorously from 9 a.m. onwards, the peak being reached between 10 and 11 a.m. It also shows considerable blooming before and after the hour of maximum flush.

The blooming in K226 was concentrated between 10 a.m. and 12 noon. During this period nearly 87 per cent. of the flowers opened, nearly 56 per cent. of the blooming was concentrated between 11 a.m. and 12 noon.

During the hours of maximum flush in the various strains temperature ranged from 82° to 93°F.

TABLE III.

Hourly bloom counts on twenty main panicles of the late Kolamba strain K42.

Date	Hours										Total florets	Percentage
	7-8 a.m.	8-9 a.m.	9-10 a.m.	10-11 a.m.	11 a.m.-12 noon	12 noon -1 p.m.	1-2 p.m.	2-3 p.m.	3-4 p.m.	4-5 p.m.		
10th Oct. 31	1	47	148	150	68	8	4	1	1	..	428	6·13
11th „ 31	14	135	618	970	245	28	14	6	3	..	2033	29·10
12th „ 31	13	21	695	1050	422	114	18	2	..	..	2335	33·42
13th „ 31	10	209	506	564	177	10	18	7	..	..	1501	21·48
14th „ 31	1	6	65	185	265	25	1	1	..	..	549	7·85
15th „ 31	2	6	4	57	72	..	..	..	..	..	141	2·01
Total florets	41	424	2036	2976	1249	185	55	17	4	..	6987	..
Percentage	0·59	6·07	29·13	42·59	17·88	2·65	0·79	0·24	0·06	..		

8. Duration of the blooming period.

The observations on blooming show that it requires from six to eight days for a strain population to finish blooming. Among the early strains, K79 and K184, a few plants begin to flower three or four days before the day of modal flowering, the number gradually increases and on the fourth and the fifth days most of the panicles are in bloom. Due to low temperatures obtaining during the month of September the blooming period is lengthened in the early strains. Among the mid-late and the late strains most of the florets open on the third, fourth and the fifth days, the largest number blooming on the fourth day.

The counts on single earheads show that a panicle requires from four to five days to complete blooming.

9. Anthesis in Kolamba rice.

Before anthesis, the enclosed anthers can be seen at the middle through the semi-transparent spikelet. Just before opening they appear to be pushed up inside the glume due to extension of the filaments. The pressure exerted by the anthers is evidenced by the gradual separation of the glume apices. The two swelling lodicules are also exerting pressure at the base of the lemma and palea.

At first, the glume apices begin to separate slowly and as the opening reaches one-third or one-half down the length of the spikelet the glumes are violently forced apart and distended at an angle of about 30 degrees. In many instances, particularly when the day is bright, anthesis is accompanied by a tiny sound. Simultaneous with the opening of the floret the anthers shed pollen partly or wholly as a result of the shock. Occasionally, however, a stray one remains tucked away in the lemma but soon it also comes out. In a few seconds the anthers assume a pendent position and whatever pollen is still adhering is blown away by wind. Devoid of pollen, the anther sacks appear whitish and turn brownish when dry.

Soon after the floret opens the bifurcated plumose stigma is seen protruding between the flowering glumes.

The glumes remain in the fully distended position for a few minutes after opening. The commencement of closing is marked by an apparent change in the position of the distended glumes. At this moment the glumes appear more or less to face each other. This change is taken to be the indication of the beginning of the closing act. The flower begins to close very slowly.

Thus, there are three distinct steps in anthesis of a rice flower, (1) time required to open, (2) time during which a flower remains *completely* open and (3) time required to close completely. Observations on all these phases were made on a total of seventy-five florets in various Kolamba strains. The data on anthesis during different weather conditions are summarized in Table IV.

TABLE IV.

Anthesis in Kolamba strains in different weather conditions during the season of 1931, at the Rice Breeding Station, Karjat, District Kolaba.

(All figures are in minutes).

Strain No.	Time taken to open completely						Time during which flower remained completely open						Time required to close completely						Total time from opening to closing					
	Bright		Cloudy		Rainy		Bright		Cloudy		Rainy		Bright		Cloudy		Rainy		Bright		Cloudy		Rainy	
	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
K79	4·28	3-6	..	..	..	..	7·35	5-12	..	..	..	..	28·71	16-37	..	..	..	..	41·00	28-48	..	..	..	..
K184	5·06	4-8	..	..	7·33	7-8	6·73	5-10	..	..	22·00	14-32	37·73	23-61	..	..	43·66	20-70	50·53	36-76	..	..	73·00	60-97
K153	5·00	4-6	7·15	4-12	10·00	10	8·50	7-10	9·84	6-17	12·00	12-00	46·00	46-00	50·07	33-78	38·00	38-00	59·50	57-62	67·00	45-105	60·00	60-00
K42	5·60	5-7	6·80	6-10	7·50	5-9	7·40	6-9	9·80	9-12	14·25	12-17	37·40	28-45	48·00	38-64	68·75	62-75	50·40	43-58	65·00	57-79	90·00	84-95
K226	5·00	5-0	6·75	6-8	9·80	9-10	5·75	5-7	8·00	7-10	15·20	13-16	33·00	28-39	39·75	33-47	58·80	48-65	43·75	38-50	54·50	47-60	84·00	70-91

It will be seen from the table above that all the three phases of anthesis are considerably influenced by climatic conditions. The various processes are hastened in bright weather, whereas a cloudy or a rainy weather considerably retards them. The rice flower requires from 5 to 10 minutes to open, depending on the atmospheric conditions. After opening, it may remain fully open from 5 to 12 minutes in a bright weather. In a cloudy or a rainy condition it may stay open as long as half an hour.

The rice flower takes longest time to close. In bright weather it may take from 32 to 45 minutes on an average. In a cloudy atmosphere it may require from 40 to 50 minutes, whereas in rainy weather the closing activity may be so delayed as to require over an hour.

V. Discussion.

The observations on the blooming of rice show that the upper branches begin to bloom first and as the panicle emerges, blooming goes on from top downwards. This agrees with the observations of Rodrigo [1925], Bhide and Bhalerao [1927] and Thadani and Dutt [1928]. The latter, however, mention that in some cases blooming commences in the middle of the panicle and continues in both upward and downward directions.

Under Karjat conditions flowers of the Kolamba rice may begin to open as early as 7-30 a.m., but such cases are few. From 9 a.m. onwards blooming increases rapidly, the most active period being from 10 a.m. to 12 noon. After this, blooming decreases rapidly and almost ceases after 2 p.m.

Only once during the twenty-four hours the rice plant reaches a maximum activity in blooming. This appears to be the experience of most of the observers. Bhide and Bhalerao [1927], however, mention that late in October when the days are warmer, flowering is in certain waves during the course of a single day. That is, blooming is revived once or more than once late in the afternoon. The authors do not present any data in support of their contention. From our experience we may say that such is not the case in Kolamba rice.

The Kolamba strains display differences in blooming. The early strains K79 and K184 show maximum blooming between 11 a.m. and 12 noon but over 28 per cent. blooming occurs *after* the modal hour in K79 whereas in K184 nearly equivalent flowering takes place *before* 11 a.m. The mid-late strain K153 shows the longest modal range, from 9 to 11 a.m., than any other strain while the late strains K42 and K226 show maximum flushes from 10 to 11 a.m. and from 11 a.m. to 12 noon respectively.

These discrepancies are a clear indication of the genetic variability existing among the strains of the Kolamba rice. Indeed, varietal differences are more pronounced as has been recorded by Ramiah [1927] and Thadani and Dutt [1928].

VI. Summary.

1. The observations on blooming and anthesis comprised of the two early strains K79 and K184, the mid-late K153 and the late strains K42 and K226 obtained by pure line selection from the Kolamba paddy, extensively grown in the North Konkan and the principal variety under improvement at the Rice Breeding station, Karjat District Kolaba.

2. The flowering in a panicle commences on the second day after emergence and proceeds from the top downwards.

3. The spikelets bloom in a regular sequence. Generally the terminal spikelet blooms first, followed by the second. After this, flowering goes on from below upwards.

4. The blooming in individual panicles is vigorous from 9 a.m. onwards and upto 12 noon. After 12 noon there is a rapid fall and hardly any flowers open after 2 p.m.

5. The Kolamba strains show differences in the magnitude of blooming at different hours.

6. A panicle completes blooming from 5 to 6 days. A strain population requires from 6 to 8 days to finish blooming.

7. The rice flower passes through three phases in anthesis, (1) time required to open, (2) time taken to remain *completely* open and (3) time required to close.

8. Atmospheric conditions greatly influence anthesis in rice. Bright weather hastens the process, whereas damp and cloudy conditions check the movements.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET

II*. PURPLE PIGMENTATION ON LEAF-SHEATH AND GLUME.

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(With Plate XLII).

Purple pigmentation is often a reliable and easily pursued index to varietal classification. The sorghum plant in spite of its stature and bodily possibilities, is not so easy a material for purple pigment to manifest itself upon, as is the case with such small cereals as rice, or Italian millet. This difficulty, notwithstanding, colour even in the vegetative portions, does help to separate the varieties.

The colour of the glume (the husk) has served as a basis for the classification of the varieties [Benson and Subba Rao, 1906], as the Tamil and Telugu names of some of the varieties will show. Vinall and Cron [1921] recorded the simple dominance of the red glume over the black. The difficulties in the grouping of the complex manifestations of this character are reflected in some of the figures given by Ramanathan [1924]. Nowhere has the sheath character, so prominent, been recorded.

In early stages when the tissues are green and sappy, differentiation in colour is not easy. But at the stage of heading and the decline in vegetative vigour, the beginnings of colour manifestation and differentiation are easily visible. Long before colour differences show in the grain or in the glumes enclosing it, the most marked evidences of differentiation are seen in the leaf-sheath. In the scanty earlier literature on the subject this easily noticeable character has not attracted the attention or care it deserves.

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* Part I.—*Ind. J. Agric. Sci.* Vol. II, part III.

Two broad types of sheath are met with. The first is the brown sheath, which occurs in plants that show no purple pigmentation on the sheath. This type is largely confined to *Sorghum Roxburghii* var. *hians*, Stapf. In this group when the sheaths start to wrinkle and dry up they put on a straw colour, with isolated patches of brown. Blotches and spots on leaves are yellowish-brown in colour.

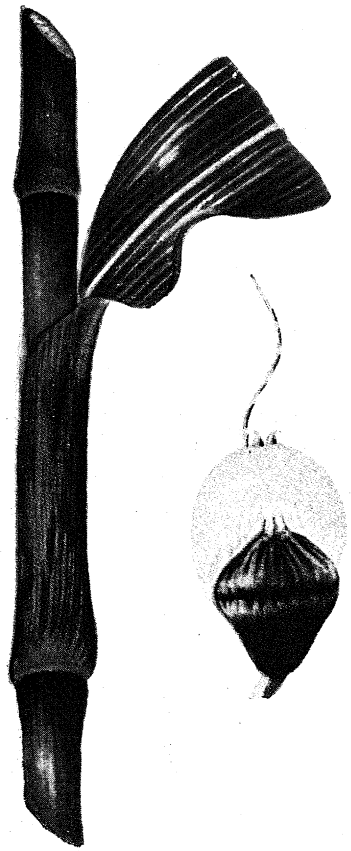
The second type is the familiar purple-pigmented sheath met with in the common *Sorghum Durra* Stapf. The presence of the pigmentation is visible in the prematurity stages, such patchiness as was referred to in the brown, taking on a purple colour. The result of this chronic patchiness is that there is practically no sheath with an even daub of purple. The leaves, when punctured or injured, run into a similar purple spottiness.

A sub-group of this purple type is singled out by the dark, practically inky tint that it takes on, as compared with the fairly bright purple of the ordinary purple sheath. Similarly, spots and patches on leaves appear blackish. There are thus three leaf-sheath colours—brown, reddish purple and blackish purple.

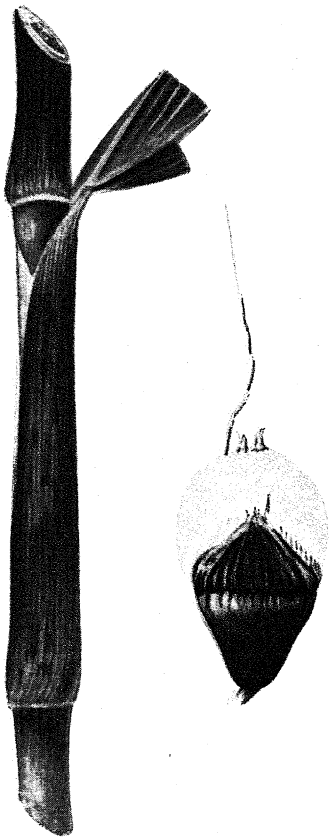
In close association with the manifestation of this pigment on the sheath and later than it in the stage of maturity, is the appearance of similar grades of colour in the glumes enclosing the rapidly maturing grain. These glumes are botanically the two outer glumes of the sorghum spikelets. The glumes are either whole and smooth and cushiony in the centre or often wrinkled up crosswise, so that as a theatre for the manifestation of colour with uniform evenness they offer a poor and inconstant surface. In non-wrinkled glumes the manifestation is very clear and glabrousness helps to set off the colour. The more obtrusive colour is the black. This occurs in plants with sheaths that are blackish purple. In purple-sheathed plants the glume is reddish. In brown sheaths the glume is brown. This parallel separation in the glumes (Plate XLII) is fairly easy with increased experience in the pursuit of this character. The greatest difficulty is introduced in the case of wrinkled glumes; this wrinkling tends to cut off the top half and desiccate it the quicker, with the result that what colour there is, lingers at the base of the glumes. So minute is the manifestation, that often the removal of the grain and a look through the hollow of the glume at the place of attachment is the only guide to the spot showing colour at the base. In the smooth non-wrinkled glumes, the central cushion, with its quick drying, often leaves a central patch of rarified colour. This difficulty makes a minute examination of the glume essential before a classification could be made.

The structure and corrugations of the glume notwithstanding, the broad fact is clear that there are three types of colour in the glumes and that these correspond and go with similar leaf sheath colours. Thus all pure lines are separable into these three groups. The sheath colour always went with the glume colour, there being no cases of the separability of this joint manifestation.

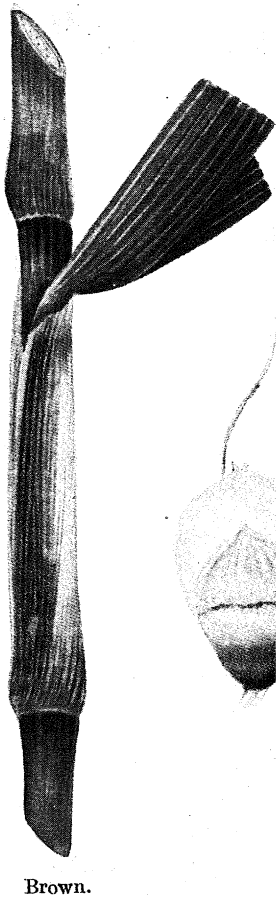
SORGHUM—SHEATH AND GLUME COLOURS.



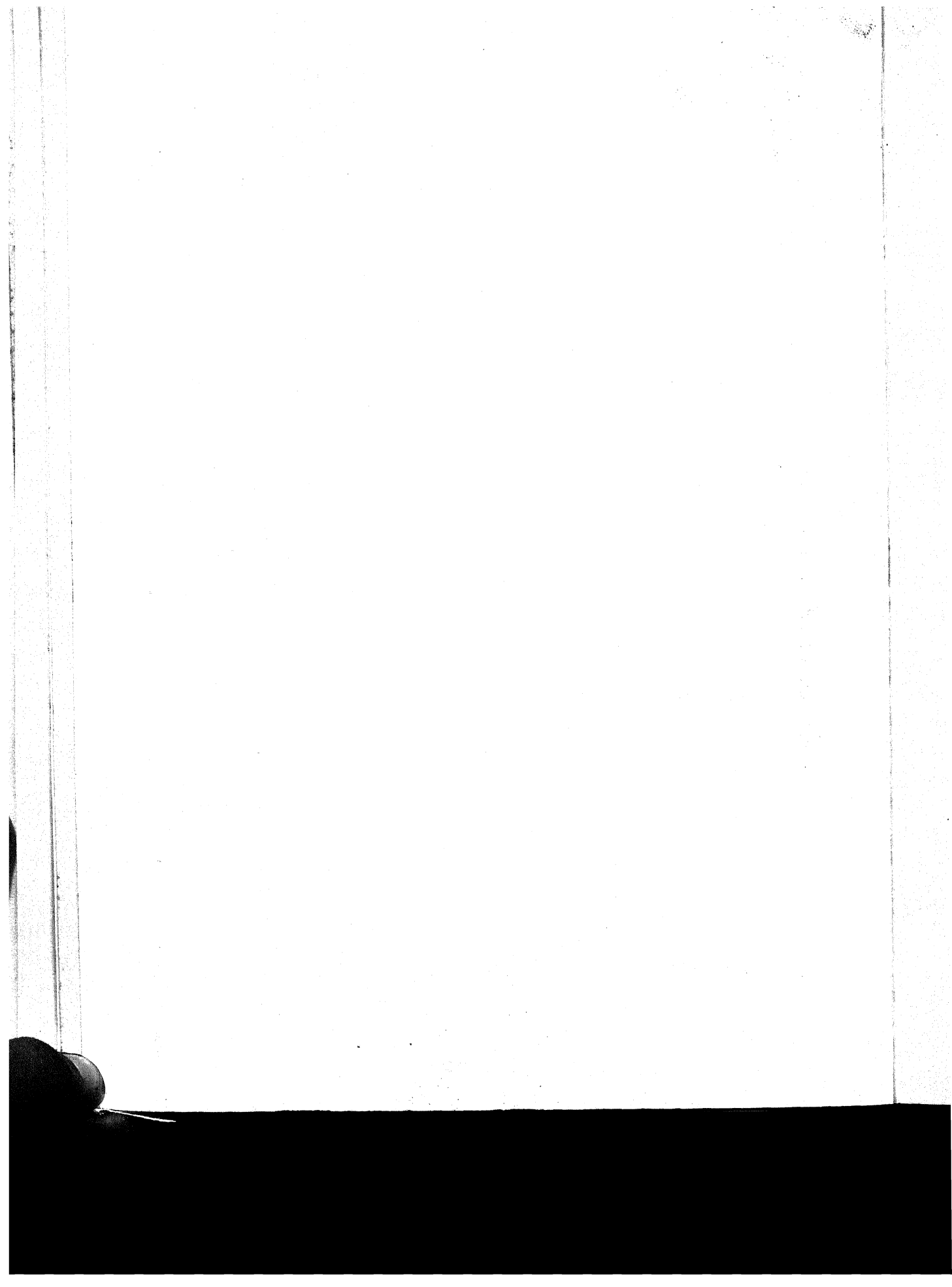
Reddish purple.



Blackish purple.



Brown.



The inheritance of this sheath-glume character, a colour character which is the most patent in the sorghum plant, has been worked out. The easy guide that the prominent sheath gives in field classification and the ready grouping that varietal names fall into, on the basis of glume colour of harvested earheads, give the pursuit of these characters in inheritance, a very practical interest.

A factor **P** is present in the purple group and absent in the browns. Purple is dominant to brown. The purple group is divisible into two sub-groups, one with a factor **Q** which makes the sheath and glume reddish purple and the other without it, which results in the sheath and glume appearing blackish purple. The former is dominant to the latter. These two factors **P** and **Q** result in the following genetic constitution of the three groups—reddish purple (**PPQQ**), blackish purple (**PPqq**), brown (**ppQQ** or **ppqq**). The inter-play of these two factors results in the usual monohybrid and dihybrid ratios. Segregations for these three sheath-glume characters are presented in the following tables. The natural crosses are the chance progenies of unbagged individuals in pure lines.

TABLE I.

Pure for **Q**, *segregating for* **P**. *Natural cross spotted in 1926.*

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours	
			Reddish purple	Brown
F_2 F_3	A. S. 1795	Reddish purple	46	17
	„ 2231	„	77	14
	„ 2233	„	76	29
	„ 2235	„	83	27
	„ 2236	„	55	13
	„ 2241	„	40	11
	„ 2232, 2238, 2239	„	Pure	—
	„ 2234, 2237, 2240, 2242	Brown	—	Pure
		Total	377	111
Other families	A. S. 396, 404, 412, 422, 474, 1157, 1158, 1304	..	557	186
		Total	934	297
		Calculated (3 : 1)	923·25	307·75

$\chi^2 = 0{\cdot}5009$, $P > 0{\cdot}3$

A. S.=*Andropogon Sorghum.*

TABLE II.

Pure for **q**, *segregating for* **P**. *Natural cross spotted in 1923.*

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours	
			Blackish purple	Brown
F_2	A. S. 426	Blackish purple	12	5
F_3	„ 462	„	100	41
	„ 463	„	95	33
	„ 1358	„	107	33
	„ 1362, 1363	Brown	—	Pure
		Total	314	112
Other families	A. S. 1376, 2546, 2547, 2552, 2555	..	145	46
		Total	459	158
		Calculated (3 : 1)	462·75	154·25

$\chi^2 = 0{\cdot}122$, $P > 0{\cdot}70$

The following data from artificial crosses confirm the experience presented above. The parents of all artificial crosses are pure lines grown for a number of years at the Millet Station.

TABLE II-A.

Artificial cross No. XXII (1927).

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours	
			Blackish purple	Brown
Parent	A. S. 1984	....	—	♀
	„ 1975	....	♂	—
F_1	„ XXII	....	Blackish purple	..
F	„ 2949	Blackish purple	212	101
	„ 2950	„	104	40
	„ 2951	„	139	56
	„ 2952	„	322	81
	„ 2953	„	280	99
		Total	1057	377
From 2nd artificial cross	A. S. 2937, 2938, 2939, 2940.		919	271
		Total	1976	648
		Calculated (3 : 1)	1968	656

$\chi^2 = 0{\cdot}13$, $P > 0{\cdot}70$

TABLE III.

Pure for **P**, *segregating for* **Q**. *Natural cross spotted in 1924.*

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours	
			Reddish purple	Blackish purple
F_2	A. S. 492	Reddish purple	63	24
F_3	,, 1442	,,	55	14
	,, 1443	,,	42	8
	,, 1444	Blackish purple	—	Pure
F_4	(From A. S. 1442)			
	A. S. 2061	Reddish purple	37	14
	,, 2062	,,	51	12
	,, 2063	,,	45	17
	,, 2064	,,	27	11
F_5	(From A. S. 2063)			
	A. S. 2575	,,	106	30
	,, 2576	,,	89	32
	,, 2578	,,	123	46
	,, 2577	,,	Pure	—
	,, 2579, 2580	Blackish purple	—	Pure
		Total	638	214
		Calculated (3 : 1)	639 :	213

$\chi^2 = 0{\cdot}0063$, $P > 0{\cdot}9$

The artificial crosses given below confirm the experience of the previous table :—

TABLE III-A.

Artificial cross No. I. (*1927*).

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours	
			Reddish purple	Blackish purple
Parent	A. S. 1842	..	♀	—
	,, 1844	..	—	♂
F_1	,, I	..	Reddish purple	—
F_2	,, 2916	Reddish purple	217	61
	,, 2917	,,	80	26
	,, 2918	,,	111	36
	,, 2919	,,	101	44
		Total	509	167
2nd cross	A. S. 2920, 2921, 2922, 2923.		381	118
3rd cross	,, 2924, 2925, 2926, 2927.		520	168
		Total	1410	453
		Calculated (3 : 1)	1397·25	465·75

$\chi^2 = 0{\cdot}465$, $P > 0{\cdot}3$

TABLE IV.

Segregating for **P** *and* **Q**. *Natural crosses spotted in 1925.*

Generation	Family No.	Character of selection	Behaviour of progeny sheath and glume colours		
			Reddish purple	Blackish purple	Brown
F_2	A. S. 1418	Reddish purple	133	56	73
	„ 1421	„	125	46	63
	„ 1423	„	74	26	38
		Total	332	128	174
		Calculated (9 : 3 : 4)	356·5	119	158·5

$\chi^2=3{\cdot}88$, $P>0{\cdot}1$

SUMMARY.

In sorghum the colour of the leaf-sheath and the colour on the glume go together. A factor **P** separates the purple-pigmented from the brown-sheathed varieties, the former being dominant. In the **P** group, a factor **Q** helps to separate the purple into reddish purple and blackish purple, the latter being recessive.

III. GRAIN COLOURS: RED, YELLOW AND WHITE.

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(With Plate XLIII)

Sorghum is a naked grain. Unlike other cereals it is not covered and protected by a husk. This exposing of the grain is the difference between the grain sorghums and the rest. In the grain sorghums the grains develop and remain prominently

out of the glume, the enclosing and keeping in position by the glumes being just at a minimum.

Grain colours are an easy guide to the classification of cultivated varieties of crops. In most cereals, grain colour is the colour of the husk, but in sorghum it is the colour of the grain itself and as such the individuality of the various colours and their inheritance is a subject of primary pursuit. The nakedness of the grain imposes the disability of exposure to various meteorological conditions and the consequent difficulty in uniform standards for the pursuit of the many colours met with. In spite of this difficulty, broad agricultural varieties with definite colours exist. The commonest colours are the favourite white and yellow. Red is not so common and brown is rare. The individuality and inheritance of the three main colours, namely, red, yellow and white are described below.

Review of literature.

Graham [1916] working on the sorghums of the Central Provinces studied the inheritance of grain colours. He classified the different groups into red, yellow and white, with a monogenic difference between any two. In the families in which all three groups occurred he found a total of 340 red-seeded, 96 yellow-seeded and 139 white-seeded plants, or a close approximation to a 9 : 3 : 4 ratio. He obtained red F_1 plants by crossing some of the whites with a yellow-grained plant, from which he assumed "that some of the whites are really undeveloped reds and only require the addition of yellow to cause the red colour to develop".

Karper and Conner [1919], and Sieglinger [1921] determined the amount of cross pollination in milo by taking advantage of the fact that the seed of the F_1 of a cross between white and yellow milo is yellow.

Conner and Karper [1923] studied the inheritance of seed-coat colour in natural crosses between Dwarf Yellow and Dwarf White Milo, Red Kafir and White Kafir, and Pink Kafir and White Kafir. In the first cross they found that yellow behaved as a simple dominant to white. The F_1 of Red Kafir × White Kafir cross was pale red, and in the F_2 a ratio of 1 red : 2 pale red : 1 white was obtained. Similar results were obtained from the cross between Pink Kafir and White Kafir.

Sieglinger [1924] made extensive studies on grain-colour inheritance and in the crosses Sunrise Kafir × Red Kafir and Red Kafir × White Kafir obtained in the F_2 generation simple monohybrid ratios of three red-seeded to one white-seeded plant.

Ramanathan [1924] obtained a ratio of three red to one white grain in the progenies of some natural crosses. In crosses which segregated for three colours he got the following numbers: 'red' 239, 'yellow' 144, and 'white' 27, and of these no explanation is given.

The senior author [Rangaswami Ayyangar, 1924], while studying the extent of natural crossing in a summar crop, observed that yellow was dominant to white and from the progenies of a number of families studied obtained a ratio of three yellow to one white in the F_2 generation.

Early monogenic experiences.

The following summarises the experiences of the earlier years (1922-25) at the Millet Breeding Station, in the segregation of grain colours. All the families are from natural crosses. It will be noticed that the segregates have been simple red to yellow, red to white, and yellow to white colours—all of the monogenic type.

Year	Family No.	Grain colours	
		Red	*Yellow*
1922	*A. S.** 137, 139, 142, 146, 419 . .	214	68
1924	„ 139, 142, 146	114	31
1925	„ 140, 141, 143, 144, 145, 518, 519, 524, 537.	698	234
		Red	*White*
1922	„ 168, 407	75	33
1924	„ 835, 836	71	23
1925	„ 319, 320, 485, 512, 514, 520, 530, 532, 534, 909.	645	202
		Yellow	*White*
1924	„ 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860. 861, 862, 863, 864, 865, 866, 867, 868, 869, 897, 898, 899.	3399	1280
1925	„ 459, 535, 932, 933, 934 . .	233	76

* *A. S.=Andropogon Sorghum.*

In the course of the examination of families *A. S.* 897, 898 and 899 quoted above an interesting relationship was noticed between grain colours and the colour of fresh anther and stigma. The segregates being yellow and white, the yellow grains had yellow anthers and yellow stigmas. The white grains had light yellow anthers and hyaline stigmas. There was an intermediate group of yellowish white grains with light yellow anthers, yellow style and hyaline stigmatic feathers, the intermediates being twice each of the pures, an interesting indication of the heterozygous condition. Thus there was segregation for fresh anther and stigma colours concurrently with grain colours [Rangaswami Ayyangar, 1925]. The above experience was from seed material drawn from the Deccan area where the meteorological conditions favour the study of these fine characters. Elsewhere this delicate character is difficult to study owing to the impossibility of securing optimum conditions for reading the character.

Early digenic experiences.

In the year 1925 the first experience of a triple segregation was met with and one of the families, *A. S.* 521, gave figures approximating the 9 : 3 : 4 ratio of red : yellow : white, referred to by Graham.

In the year 1926 enough of this triple segregation was noticed and the 9 : 3 : 4 ratio was met with *in extenso*. The sum total of this is given below :—

Year	Family No.	Grain colours		
		Red	Yellow	White
1926	A. S. 1011, 1150, 1157, 1158, 1163, 1314, 1334, 1337, 1339, 1340, 1341, 1342, 1343, 1344, 1409, 1498, 1499, 1500	1029	292	404

Differentiation of the 'whites'.

The reason given by Graham that certain white-grained plants are undeveloped reds requiring the presence of yellow to cause the red colour to develop not being sufficiently explanatory, a close examination of the white group was made. At the

stage of flowering no differences were noticeable. But when the anthesis was over and the greenish growing grain was emerging, the mass of dry anthers clinging to the earhead showed a differentiation between plants and looked like giving an indication to the composition of the whites [Rangaswami Ayyangar, 1926]. The optimum conditions for regular counts did not exist but the incidence was distinctly of a type that showed a mixture of plants with dry anthers coloured red and sienna, the former being quite in excess. This gave the clue for the study of this fine difference in the whites of the triple segregates, and a similar occurrence of the two sets of dry anthers, *viz.*, very light red and light sienna, was noticed in that group. When harvested heads are tapped on a piece of paper the dry anthers clinging to the crevices fall down and can be classified. But it should be noted that this character is best studied and recorded when the anthesis is in full swing and when the earhead is covered by a mass of dry anthers with a sprinkling of fresh ones.

This certain occurrence of the two kinds of dry anther colours in the white grain group was studied in the harvested heads by a close examination of every part of the husk and grain and led to the detection of differences in the base of the husked grain, most of them having a zone of colour round the place of attachment in the area covered by the glumes [Rangaswami Ayyangar, 1927, 2].

The result of the examination of the grain bases in the white heads of the triple segregates are given below :—

Year	Family No.	Grain colours, white grain	
		with red base	with yellow base
1926	A. S. 1067, 1177, 1178, 1211, 1212.	91	29

It will be obvious from the above table that there was a segregation for the basal colour of the white group and that presumably the dry anther segregations were the forerunners of this. Thus the need for the study of both the dry anther colour and the colour of the grain base was clearly indicated and in subsequent years studied. Along with the above two characters, the closely related fresh anther and stigma colours have also been noted [Rangaswami Ayyangar *et al*, 1933].

The histories of clans *A. S.* 840 and *A. S.* 1157 pursued through generations are given in the following tables:—

TABLE I.

A. S. 840 clan. Natural cross spotted in 1923.

Generation	Family No.	Character of selection (grain)	Stigma -- Anther fresh -- Anther dry -- Grain --	Behaviour of progeny			
				--- Y ---	--- Y ---	---VLY---	---VLY*
				--- Y ---	--- Y ---	---VLY---	--- VLY
				--- R ---	--- S ---	---VLR---	----LS
				---Red---	--Yellow--	--White-- (R. base)	--- White (Y. base)
F_2	A. S. 840	Red	..	30	13		..
F_3	,, 922	,,	..	39	13	10	..
	,, 908	,,	..	37	..	11	..
	,, 909	,,	..	53	..	15	..
	,, 921	,,	..	pure	..	..	..
	,, 923	Yellow	..	..	pure	..	..
	,, 924	White (R. base)	..	..	..	pure	..
F_4	(from A. S. 922)						
	A. S. 1163	Red	..	71	19	30	9
	,, 1162	,,	..	104	28	..	..
	,, 1164	,,	..	118	31	..	..
	,, 1165	,,	..	112	..	38	..
	,, 1166	,,	..	114	..	31	..
	,, 1167	,,	..	105	..	43	..
	,, 1168	Yellow	..	..	103	..	30
	,, 1169	,,	..	..	109	..	32
	,, 1170	,,	..	..	107	..	23
	,, 1171	,,	..	..	96	..	41
	,, 1172	,,	..	..	115	..	31
	,, 1174	White (R. base)	..	..	..	92	20
	,, 1173, 1175	,,	..	..	..	pure	..
	(from A. S. 908)						
	A. S. 1216	Red	..	52	..	26	..
	,, 1217	,,	..	58	..	20	..
	,, 1218	,,	..	64	..	17	..
	,, 1215	,,	..	pure	..	..	..
	,, 1219, 1220	White	..	..	..	pure	.
F_5	(from A. S. 1164)						
	A. S. 1621	Red	..	27	10	..	..
	,, 1623	,,	..	29	9	..	..
	,, 1622	,,	..	pure	..	..	..
	,, 1624	Yellow	..	..	pure	..	..
	(from A. S. 1165)						
	A. S. 1625	White (R. base)	..	..	..	pure	..
	(from A. S. 1174)						
	A. S. 1627	,,	..	..	..	59	24
	,, 1629	,,	..	..	..	51	12
	,, 1628	,,	..	..	..	pure	..
	,, 1630, 1631	White (Y. base)	..	..	..	..	pure
	(from A. S. 1168)						
	A. S. 1626	,,	..	..	..	..	pure

* Y=Yellow, R=Red, S=Sienna, V=Very, L=Light.

TABLE II.

A. S. 1157 clan. Natural cross spotted in 1925.

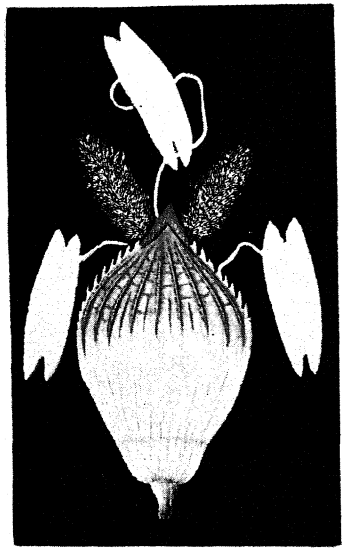
Generation	Family No.	Character of selection (grain)	Stigma---Anther fresh---Anther dry---Grain	Behaviour of progeny			
				---Y--- / ---Y--- / --VLY-- / ---VLY			
				---Y--- / ---Y--- / --VLY--- / ---VLY			
				---R--- / ---S--- / --VLR-- / ---LS			
				---Red--- / --Yellow-- / --White-- (R. base) / --White-- (Y. base)			
F_2	A. S. 1157	Red	..	77	21	20	
F_3	,, 1609	,,	..	21	5	5	2
	,, 1610	,,	..	15	8	7	2
	,, 1608	,,	..	34	14	..	..
	,, 1611	Yellow	..	..	123	..	46
	,, 1612	White (R. base)	..	..	..	35	14
	,, 1613	,,	..	..	..	40	14
	,, 1614	White (Y. base)	..	..	..	..	pure
F_4	(from A. S. 1608)						
	A. S. 2133	Red	..	85	27	..	..
	,, 2134	,,	..	83	20	..	..
	,, 2136	,,	..	85	26	..	..
	,, 2135	,,	..	pure	..	..	..
	(from A. S. 1161)						
	A. S. 2488	Yellow	..	..	38	..	10
	,, 2489	,,	..	..	73	..	17
	,, 2490	,,	..	..	31	..	2
	,, 2487	,,	..	..	pure	..	..
	(from A. S. 1613)						
	A. S. 2138	White (R. base)	..	..	..	82	18
	,, 2139	,,	..	..	..	86	29
	,, 2140	,,	..	..	..	89	23
	,, 2137	,,	..	..	..	pure	..
	,, 1614	White (Y. base)	..	..	..	..	pure

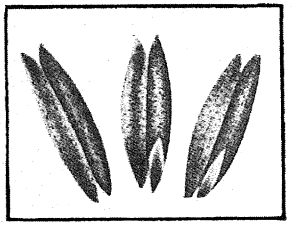
Other experiences of grain base segregation have been met with in 15 families with a total of 738 red base to 259 yellow base.

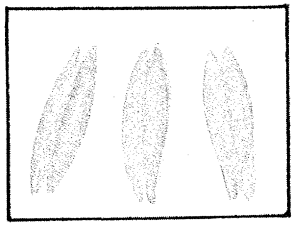
GRAIN BASE COLOUR AND GAPING GLUMES.

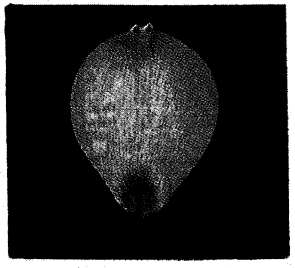
While discussing the subject of basal colour of white grains it is necessary to note that this appearance of colour is consequent on the protection afforded by the glumes against the bleaching of even this vestige of colour.

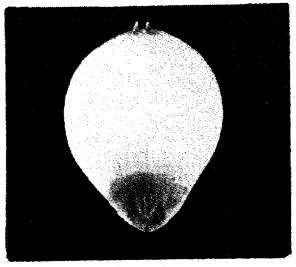
In *Sorghum Roxburghii* var. *hians*, the glumes in mature grains gape out and expose grain bases. With this character of gaping glume segregates have been met with for grain colour in which the white group of grains could not be separated on the basis of grain base colour into red base and yellow base. That

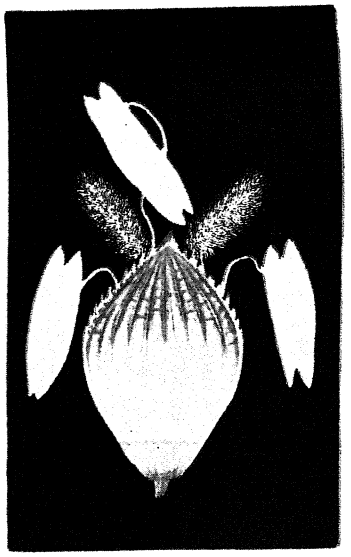


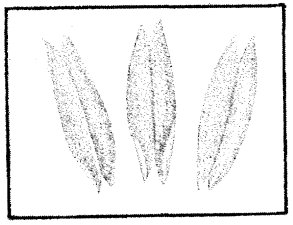


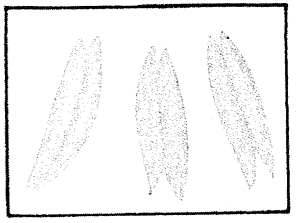


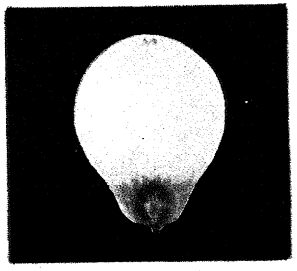


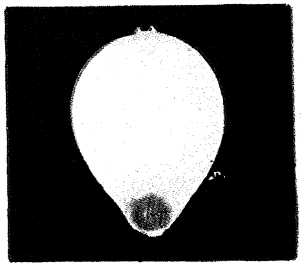












this non-differentiation was only an effect of the gaping glumes exposing grain bases will be clear from the following segregations for dry anther colour alone met with in the white grain group of families with gaping glumes.

	Grain white—no basal colour	
	Dry anther segregations	
	Very light red	Very light brown
A. S. 1914, 1915, 1926, 1930 . . .	182	68

Discussion.

R *and* W *factors.*

The foregoing data is unmistakable in its import. That there is a single factor, the addition of which to the yellow results in red is obvious. The innumerable monohybrid segregations for red and yellow prove it. The classification of the whites on the basis of the colour of their bases is also definite. The patch of red colour is confined to the base under the protection of the glumes. The conclusion is unmistakable that in the red-based white the factor that could produce the colour all over the grain is lacking [Rangaswami Ayyangar, 1927, 1]. These whites are reds with the disability to manifest whole colour. The white is the result of the elimination of the factor for whole colour. Graham's supposition that the yellow colour has some connection in activating some of the whites to produce red has to be interpreted not as a direct contribution through the yellow but as the coming in of the factor for the 'wholeness' through the 'whole yellow'. On this interpretation both the reds and the yellows have a factor **W** that results in their ability to put on whole colour—red or yellow. In the absence of **W**, ths colour is confined to the base. The collateral effect of the presence of this **W** factor is reflected in the deeper tint of the fresh anther in the whole coloured reds and yellows and in correspondingly lighter tints in the whites, whether red or yellow based (Plate XLIII). The single factor difference recognised by all previous workers, namely **R**, between red and yellow has its effects in the colour of the dry anther so that wherever red appears, be it whole or basal, the tint is red, and wherever there is yellow, be it whole or basal, the dry colour is sienna.

In artificial crosses Nos. *A.S.* LXXV and *A.S.* LXXIX made between yellow grain and red-based white grain, the F_1 plants were whole red, as was expected.

Y *factor.*

The anthers of all the sorghums so far examined are yellow in colour when fresh. They differ from each other only in the depth of the yellow colour. All anthers when dry have a basic yellow colour, though withering imparts to the lowest depth of colour of these dry anthers a sienna tint. From this sienna tint they grade up in depths of brown when factors for brown are present. This

ramification consequent on the presence of brown factors will be dealt with separately. When there is red the colour is so overpowering that the yellow is masked as well as the brown, so much so that the red dry anthers will grade up in depths according to the basic yellow or brown in them.

Sections of the sorghum grains in the many varieties examined reveal a tint of yellow in the aleurone layer of practically every one of the types, including the whites. This yellow in the aleurone layer, corresponding to the yellow in the anther fresh and dry, seems, therefore, to be a fundamental attribute of the sorghums of the hot climates in their natural habitat. A factor **Y** is therefore taken as the starting point for the colour schemes in the sorghum grains.

I *factor—pink grains.*

We have thus three factors **R**, **W**, and **Y**. Closely acting on these factors is a factor **I** that determines the manifestation in intensity of the above colour factors. As has been said already the maturity of the grains in open imposes difficulties in the separation of shades in colours, but in the obtrusive red the effect of the **I** factor is very patent giving rise to two distinct types of grain, red and pink. The pink grain is characteristic in its translucence, a veneer of red showing over a yellow background. In the foregoing tables the red-yellow-white segregation have been given with the **I** factor common to all. The following table summarises a similar experience without the **I** factor, giving pink, yellow and white segregations :—

Family No.				Grain colours		
				Pink	Yellow	White (R. base)
A. S.	926,	1179,	1180,			
	1182,	1183,	1192,			
	1194,	1655,	1657,			
	1658,	1659,	1661.	788	240	..
A. S.	369,	1027,	1029,			
	1566,	1567,	1568,			
	1573,	2094.		326	..	84

In the following table the **I** factor is at play :—

Family No.			Grain colours		
			Red	Pink	White (R. base)
A. S.	183,	1161,	136	44	55
,,	1024.		74	25	..

In the presence of **R**, the **I** factor is thus easily at play. Yellows with and without the **I** factor are difficult of clear separation. The lightness of the colour and the quick reaction to weather conditions coupled with a brown wash found in most yellow varieties, add to this difficulty. Even so with the help of the **R** factor, the yellow allelomorphs could be separated. **A. S.** 812 is a pure line with white grains having red bases—**R** without **W**. Two yellows **A. S.** 810 and **A. S.** 817, were crossed with it. The F_1s from the former were red-grained and from the latter pink. The difference between the yellow was so faint that but for this noticeable behaviour with **R**, the two yellows would have passed undifferentiated. This nicety between them represents the effect of the **I** factor.

Thus we arrive at the following genetic constitutions for the red-yellow-white group of grain colours :—

Red	**YY RR WW II**
Pink	**YY RR WW ii**
Yellow	**YY rr WW II** **YY rr WW ii**
White (red base)	**YY RR ww II** **YY RR ww ii**
White (yellow base)	**YY rr ww II** **YY rr ww ii**

Summary.

The inter-relationships between red, yellow and white grain colours in sorghum have been pursued. Yellow (factor **Y**) is the basic colour. With **R**, red grains are produced. A factor **W** determines the manifestation of colour in wholeness. Without **W**, **R** gives a white grain with a red base; similarly **Y**, gives a white grain with a yellow base.

Dry anther colours run parallel to grain colours and help in the separation of the white grains into their respective allelomorphs to coloured grains.

A factor **I** determines the intensity of colour manifestation and is unmistakably noticeable in the red group. Red without the **I** factor gives a pink grain.

Monogenic and digenic interactions of these factors have been met with.

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STUDIES IN SORGHUM—THE GREAT MILLET.

II.* ANTHER, STIGMA AND GRAIN COLOUR AFFINITIES.

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(With Plate XLIV).

The sorghum plant is slow to develop purple pigmentation. With the beginning of the reproductive phase the latent possibilities of the plant for this manifestation begin to show, but with the disability that the scope for its manifestation is getting reduced and that even this is subject to the depredations of the various meteorological conditions. The glumes do not show much colour development in the very early stages of the heading and it is often not till well after pollination that glume colour starts to develop. Grain colour does not show best till the head is just ready for harvest. In a family segregating for characters apart from such a patent character as awn, it is difficult to judge the nature of a head, as the glumes in most cases show little apparent differentiation. It is therefore necessary to look out for all recognizable characters that could aid selection of plants for selfing in the early stages and later on to serve as checks to fix up grain groups, whose sharp classification is often rendered difficult by the fact of their maturity in the open. With the beginning of anthesis and the concurrent protrusion of the stigmas waiting to be pollinated, varietal differences can in a way be marked out [Rangaswami Ayyangar, 1925]. Graham [1916] mentions that the colour of the plant can be detected at the time of flowering, the stamens and stigmas of the red-grained plants being orange, and those of the yellow and white pale yellow.

Stigma.

Stigmas and their feathers are usually yellow in colour. In the deeper coloured grains there is a tendency for the stigma to appear in deeper shades of yellow

* Part I.—*Ind. J. Agric. Sci.*, Vol. I, Part IV.

when fresh. In the lighter shades of grain colour and in the big group of white grains, the stigma lightens its colour both in depth and in distribution so that stigmas that are hyaline and others in which the yellow tint is confined to the central axis or to stray feathers of the stigma, are met with. Definite grouping can within limits be attempted, but for the fact that the delicacy of the organ concerned, its quick withering, liability to mould, and a smothering with pollen and response to colour reactions on withering, add to the difficulties of clear-cut grouping. Even so, the following groups can be made:—deep yellow, yellow, light yellow, and very light yellow, running down to colourless hyaline stigmas.

Fresh anthers.

Parallel to this grouping in the stigma, and much more definite and individualistic, and considerably easier in pursuit is the classification of the fresh anther. Among freshly emerging anthers the following groups occur:—deep yellow, yellow, light yellow, and very light yellow. No white anthers have so far been met with.

The defect in the parallelism between the stigma and the fresh anther colours raises a doubt as to whether some extreme forms of light yellow in the anthers are really whites or whether the hyaline stigmas have any elements of yellow in them not easily noticeable—problems that await clarification with further observations.

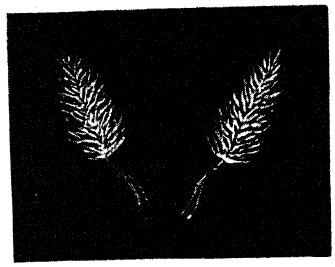
Pollen grains.

The more delicate pollen grains show differences in the depth of yellow. Red- and yellow-coloured grains have yellow pollen, the former being a shade deeper in colour. White-grained varieties have pollen grains coloured light yellow.

Stigma and fresh anther relations.

How these coloured contents of the fresh anthers contribute to the difficulty of fixing the lower grades of anther colour, is the measure by which the absence of white anthers could be explained. The fresh stigma and the fresh anther therefore fall into the following groups (Plate XLIV):—

Fresh stigma	Fresh anther
1. Deep yellow	Deep yellow
2. Yellow	Yellow
3. Light yellow	Light yellow
4. Very light yellow	Very light yellow
5. Hyaline	Light yellow Very light yellow



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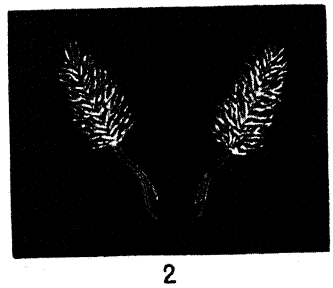

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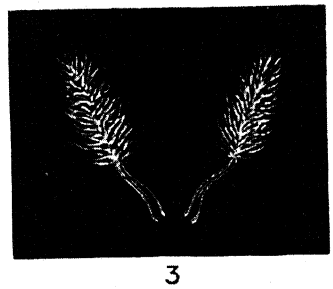

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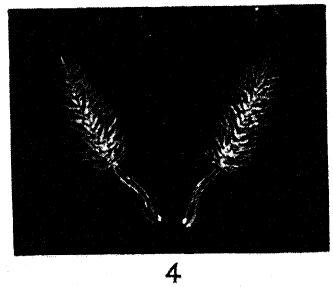

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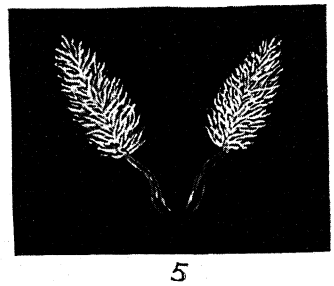

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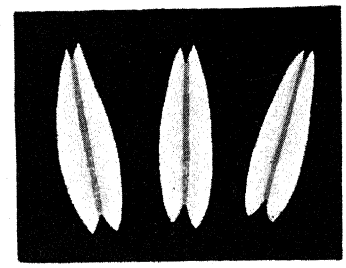

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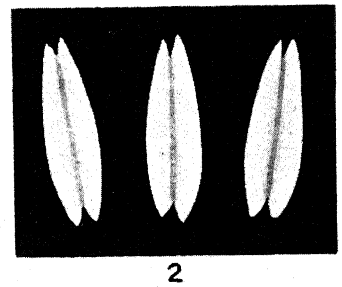

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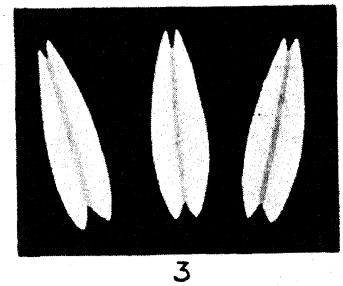

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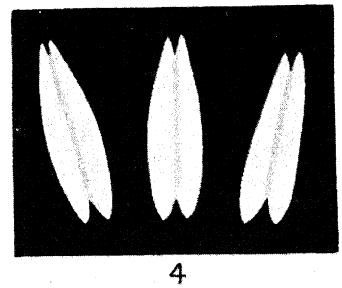

4

3 or 4

Dry anthers.

The anther sacs when they dry put on various colours. Patel [1926] utilized the weathered colour of the anther to spot natural crosses. Patel and Patel [1929] record that all the distinctly red anthers had also the shrivelled-up stigmas of a red colour. In the case of yellow-anthered types the stigma was likewise whitish yellow to yellow in colour. The dry anther colours have received attention at the Millet Breeding Station from early years and the relationship of dry anther colours to grain colours has been recorded [Rangaswami Ayyangar, 1926 and 1927]. There is a rough parallelism between these colours and the colours that appear later on on the grain. It is the differential response that these dry anthers show that helps in the separation of the white grains into sub-groups. A white grain is the result of negation of colour factors, or absence of the faculty for the manifestation of colour. This absence of the factor for manifestation is reflected in the subtle hyaline stigma which is the monopoly of the white grain group.

Dry anthers fall under three broad colour groups—brown, red, and sienna (yellowish). The sienna dry anther ranges from a very light buff to sienna that runs the risk of merging into the lighter shades of the brown group. The sienna is definitely different from the brown in that it lacks the factor for brown. The mass effect of brown is evident in the brown grain group, but in the sienna only stray brown colouration consequent on weathering is met with. The most individualistic group is the red. The brightness of the red tint dominates other basic or superimposed colours so much so that the presence of the red either over the sienna or under the brown stands out clearly. As has been said above, the whites vary in their composition. There are whites related to the brown, to the red, and to the yellow, so much so that the white-grained dry anther group gives a range from brown through red to sienna with a blend of shades.

Grain colours.

In dealing with grain colours, since we have to parallel the anther colours, it is best to set aside the white grains whose individuality depends upon their coloured relations. It can be said generally that yellow grains have sienna dry anthers, red grains red dry anthers, and brown grains brown dry anthers. The inter-relationship between the brown, red and yellow and the genetic composition of the groups among browns, among reds and among the yellows together with the existence of a factor or factors governing the blend of colours give rise to various qualifications, like very light, light, reddish, brownish, etc., modifying and increasing these basic colour groups. These sub-groups are best handled concurrently with grain colours and their inheritance.

SUMMARY.

Fresh anther colours vary in the tint of their yellow from deep to very light. These correspond to similar gradations of colour on the stigmas. Some of the stigmas are hyaline and in these the anthers may be light yellow or very light yellow.

Pollen grains vary in colour and in the deep-coloured grains are deep yellow and correspondingly lighter in the lighter-coloured grains.

Anthers when dry become coloured, the colours appearing earlier and parallel to the later developing grain colours. White grains could be classified into sub-groups on the basis of their dry anther colours.

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BIOMETRIC STUDIES IN SORGHUM.

THE RELATION OF YIELD TO OTHER CHARACTERS IN *ANDROPOGON SORGHUM*.

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(With seven text-figures).

INTRODUCTION.

To the plant breeder who is often confronted with a diverse variety of plants and types from a number of sources, the problem of their economic significance is a subject of much fascinating study. The nature and configuration of the several types, their behaviour under different conditions and their relation to yield are all of fundamental importance in the handling of crops. Towards this end, character studies with the yield as the basis, have a natural bearing. The result of such a study, made on the '*pacha*' *jonna*, a variety of *Andropogon Sorghum* extensively cultivated in the Nandyal valley of Kurnool district, are presented in this paper.

MATERIAL AND METHODS.

The plants examined comprised 34 pure lines of *pacha jonna*, being the progeny of single plants originally selected by Mr. G. N. Rangaswami Ayyangar, the Millet Specialist, Coimbatore, and his assistants and grown at the Nandyal Agricultural Research Station during the year 1931-32. Due to severe attack of the earhead bug, *Calocoris angustatus*, observations were confined to healthy selections in each of which 50, or sometimes more, good plants were examined. This material was supplemented by measurements on 450 plants in one field and 300 in another, on an *n*th generation bulk of T. 6, a strain of *pacha jonna*.

The characters included for study were as follows :—

(1) Weight of head (grams)
(2) Circumference of head (centimetres)
(3) Diameter of peduncle (centimetres)
(4) Length of head (centimetres)
(5) Height of plant (centimetres)
(6) Length of peduncle (centimetres), and

(7) Emergence (centimetres), the last character being measured by the distance from the flag junction to the base of the head, being positive or negative, according as this base is above or below that junction.

CORRELATION OF THE DIFFERENT CHARACTERS WITH THE YIELD OF THE PLANT.

As a first step in the enquiry the relations of yield to other characters were studied by the usual method of correlation coefficients of Galton, taking as the basis of yield the weight of the earhead. The correlations were determined in three ways, namely—

(1) By the value of the coefficients in moderately large samples, and in different fields, of one strain;

(2) By their distribution in small samples in a number of strains; and

(3) By the correlations between the means of different strains.

As the results got by all these methods were, more or less, similar in essentials, they are together presented in a condensed form in Table I.

TABLE I.

Correlation coefficients of yield with other characters in bulk, within strains and between strains.

Subject: Weight of head (I)				
Column (I)	(II)	(III)	(IV)	(V)
Relative	T_6-Bulk, Field 2B. (457 Plants)	T_6-Bulk F. 7. (300 Plants)	In 34 different strains mean r	Between means of 34 pure strains
Circumference of head (2)	·9681±·0019	·8425±·0112	·908±·006	·6544±·0663
Diameter of peduncle (3)	·8694±·0077	·7596±·0163	·807±·007	·6895±·0553
Length of head (4)	·7862±·0119	·5659±·0262	·705±·013	·1179±·1143
Height of plant (5)	·5578±·0215	·2936±·0352	·517±·023	—·0025±·1161
Emergence (6)	—·3946±·0264	—·3070±·0349	—·234±·023	—·3112±·0959
Length of peduncle (7)	—·1365±·0307	—·2150±·0367	·040±·023	—·4166±·1048

Taking first the relation of yield to other characters within the same strain (columns II, III and IV in Table I) it is seen that the characters most related to yields are in the order of importance, (1) the circumference of head, (2) diameter of peduncle, (3) length of head and (4) height of plant, the correlation in all these cases being positive. The emergence, however, shows a much smaller but significantly negative effect, while the relation of length of peduncle is still less, being sometimes insignificant. The actual distribution of the thirty-four correlation coefficients pertaining to Column IV is given in Fig. 1.

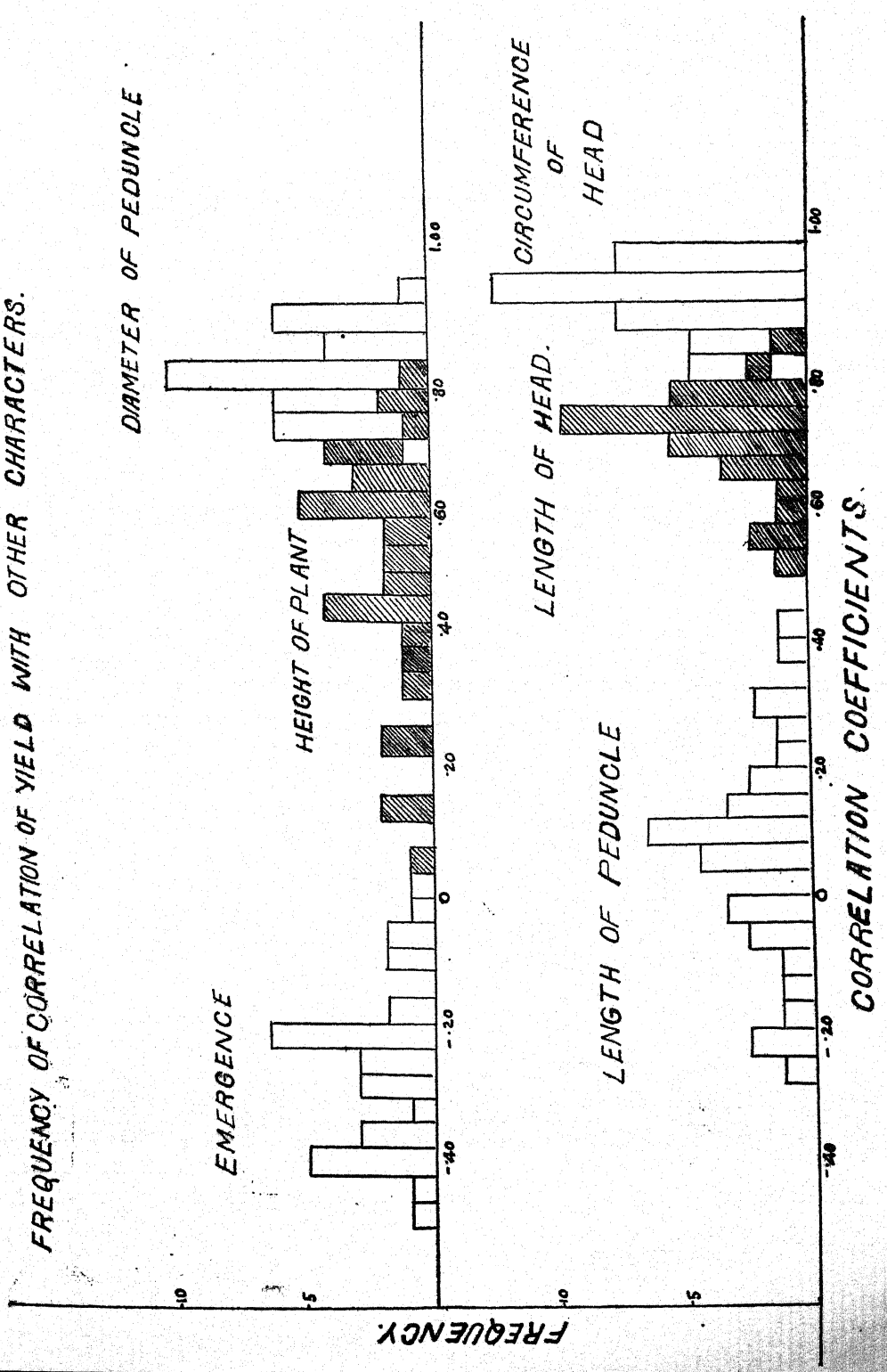
FREQUENCY OF CORRELATION OF YIELD WITH OTHER CHARACTERS.
DIAMETER OF PEDUNCLE
HEIGHT OF PLANT
EMERGENCE
CIRCUMFERENCE OF HEAD
LENGTH OF HEAD.
LENGTH OF PEDUNCLE
FREQUENCY
CORRELATION COEFFICIENTS.
10
5
0
-.20
-.40
.20
.40
.60
.80
1.00

FIGURE 1

It will be seen that even though the number of plants examined was so small as fifty, the distributions of the correlation coefficients are fairly consistent, while the result has been further confirmed by the larger sampling (Columns II and III). It can be inferred from this that in plants within the same strain the circumference of head, diameter of peduncle and length of head play an important role in the determination of yield and to a less extent the other characters. The extent to which these relations of the characters are affected in their movements through the different strains is given by the following analysis of variance.

TABLE II.

Correlations of characters : anylysis of variance.

Variance due to	Freedom	Sum of squares	Mean square
Between characters	5	36·3632	7·27260
Between pure lines	33	1·6506	0·05002
Random	165	3·2266	0·01956
Total	203	41·2404	

Considered in respect of their half logarithmic values, the variances due to both causes, the characters as well as the pure lines, are significant ($P<·01$), the former very highly so. Since the contribution from the character differences is very large, it can be inferred that the relationships (Column IV, Table I) are generally true of *'pacha' jonnas* as a whole. There exists however another source of variation associated with genetic causes, which makes some particular selections behave differently from the rest in their character relations. This effect is comparatively small though significant, as can be seen by the variance due to pure lines.

GENETIC CORRELATIONS.

The relations above discussed refer to the correlations of characters within plants of the same strain. The next step will be to inquire how far they vary from one selection to another. For this purpose the correlations between the means of different strains were investigated. The results of such a study are given in Column V, Table I.

It will be seen that the relationship between strains is not the same as that of plants within the same strain. As before, the correlations of circumference of head and diameter of peduncle are high but not as high as within the same type. The

length of head or the height of plant of a particular selection seem to have no special significance towards yield, and this is just in contrast to the plant relations. On the other hand selections with a long peduncle, or high emergence are again poor yielders. Although in selection work a freely emerging type of head is preferable on account of the freedom from disease and from the harmful effects of rain associated with it, it does not seem desirable to choose for this character beyond the required limits as otherwise smaller heads will be got. Considering that these correlations have been got from mean values where the variability has been much reduced particular significance attaches to the result. It can be concluded from these that the yield of the plant exhibits a marked relation to the other characters from plant to plant of the same type and that this relationship is true but in a modified form and different degrees from one type to another. The next step in the enquiry will be to study in what manner these influences make for the yield.

Regression of yield on the other characters.

For the above purpose, changes in yield brought about by changes in each of the other characters were investigated. Moderately large samples of plants were examined in two different fields and the relations discussed in this and the succeeding section refer to variations of plants within the same type, which are associated with environmental causes. They are presented in Figs. 2-7.

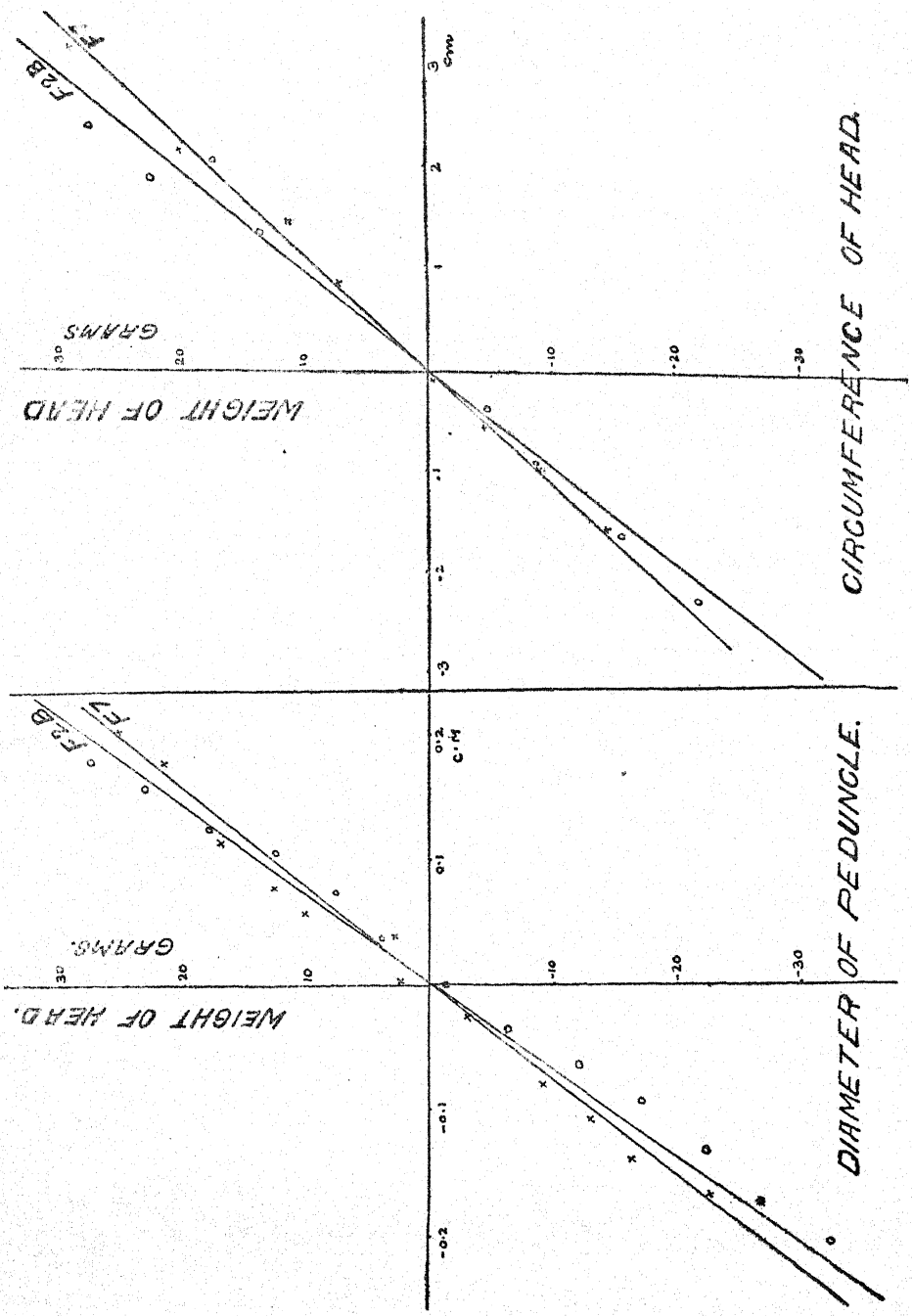

Fig. 3.—Relation of weight of head to circumference of head.

Fig. 2.—Relation of weight of head to diameter of peduncle.

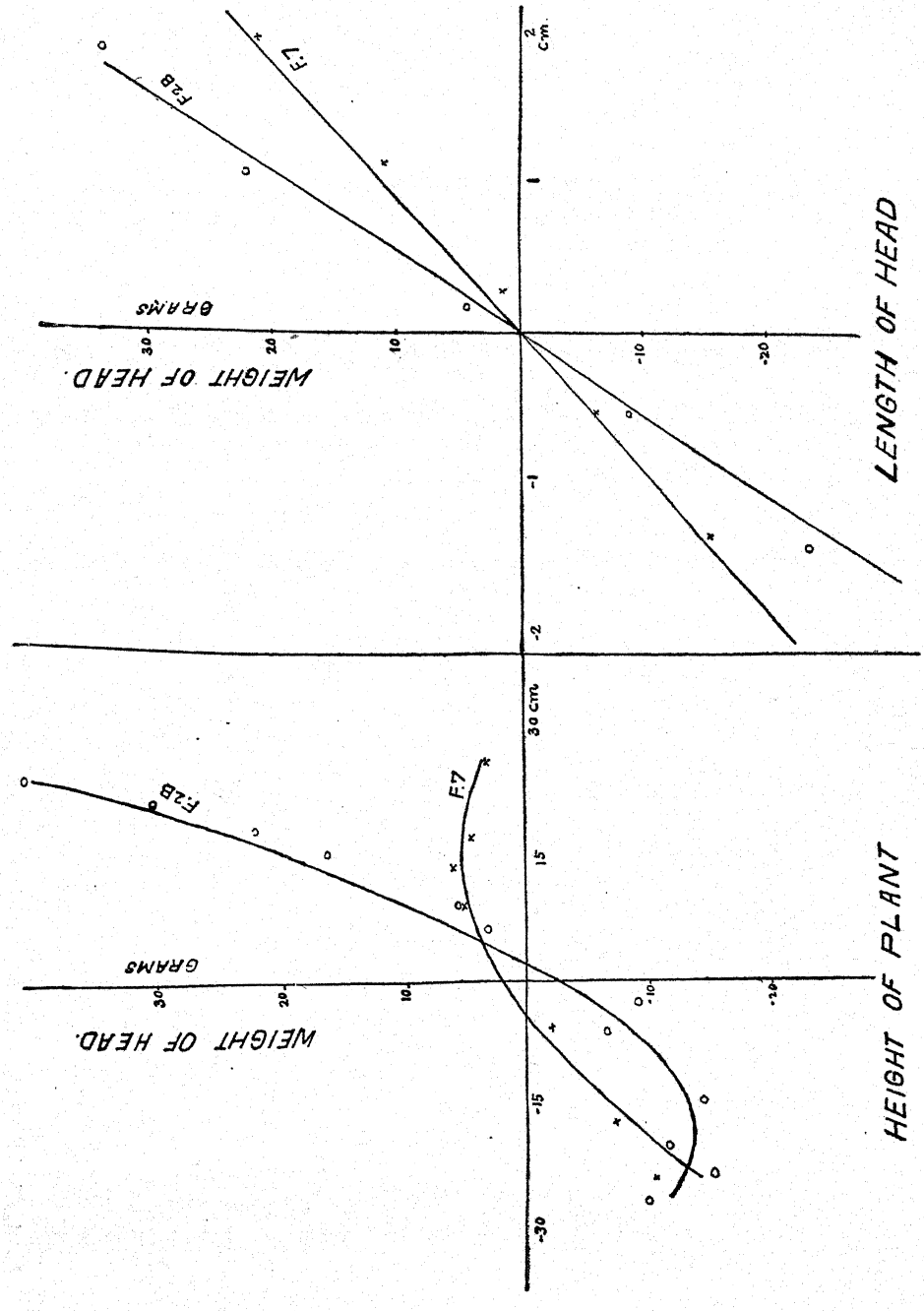

Fig. 5.—Relation of weight of head to length of head.

Fig. 4.—Relation of weight of head to height of plant.

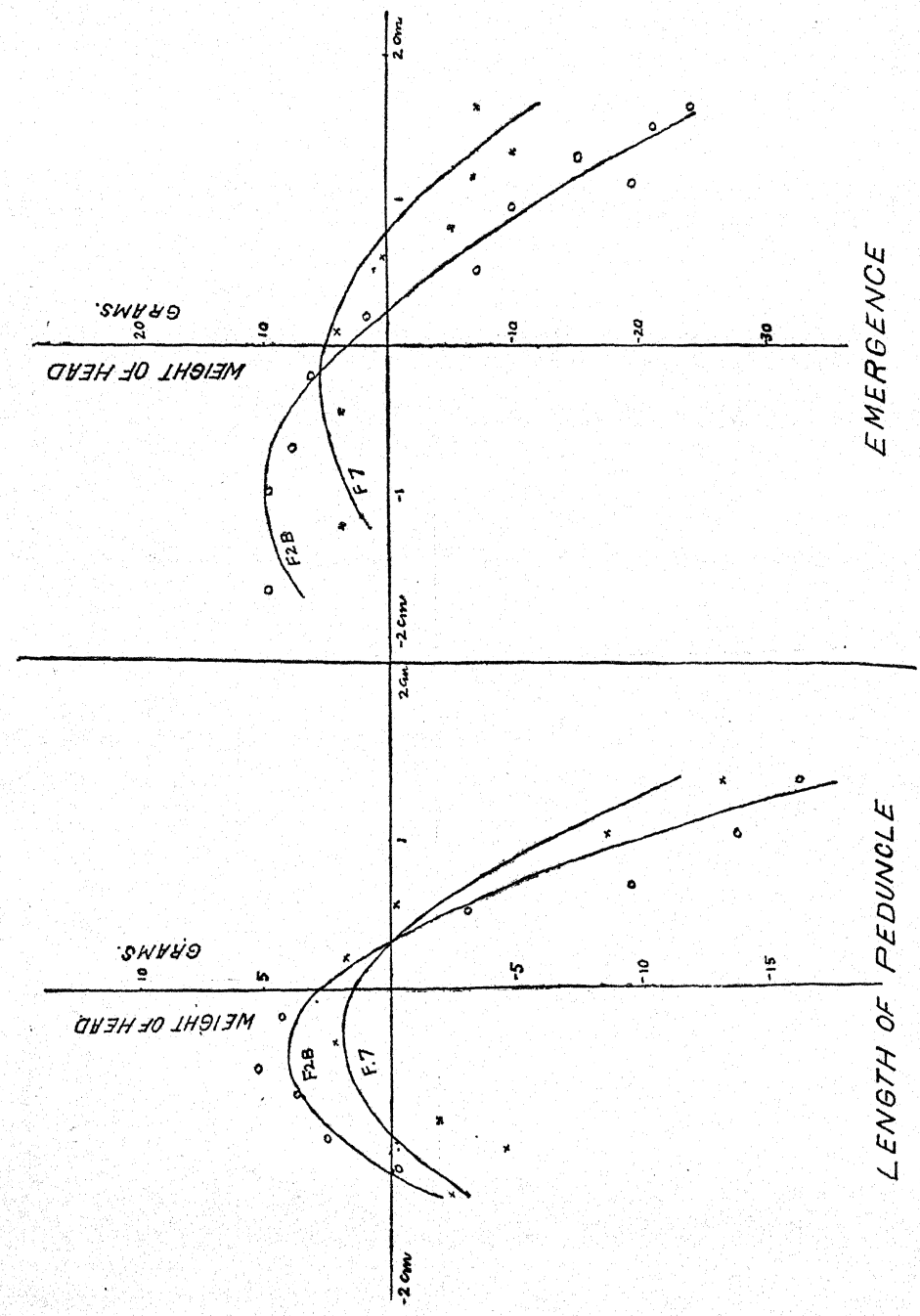

Fig. 6.—Relation of weight of head to length of peduncle.

Fig. 7.—Relation of weight of head to emergence.

An examination of these figures shows that in the characters circumference of head (Fig. 3), diameter of peduncle (Fig. 2) and length of head (Fig. 5), the relation is very nearly a straight line. The extent to which the regressions are accounted for by rectilinear variation was determined by Fisher's [1930] test of significance given below :—

TABLE III.

Test of straightness of regression lines.

Variance due to	Circumference of head			Diameter of peduncle			Length of head		
	Freedom	Sum of squares	Mean square	Freedom	Sum of squares	Mean square	Freedom	Sum of squares	Mean square
Field 2-B.									
Within arrays	434	2734	63·0	434	44151	101·7	450	96441	214·3
Linear regression	1	206705	206705	1	166905	166905	1	124256	124256
Deviation from linear regression	21	13164	626·9	21	11547	547·7	5	1906	381·2
Value of '*P*' for linear regression	(Significant)<·01			(Significant)<·01			(Not significant)		
Field 7									
Within arrays	285	15845	55·6	284	22389	78·8	295	38877	131·8
Linear regression	1	39593	39593	1	32248	32248	1	17806	17806·0
Deviation from linear regression	13	1666	128·2	14	2467	176·2	3	421	140·3
Value of '*P*' for linear regression	(Significant)<·05			(Significant)<·05			(Not significant)		

It was found that while in the characters circumference of head and diameter of penduncle the departure from a straight line was significant, the length of head gave a very good fit. It was also noted in all of these characters that the variance due to rectilinear regression was very large compared to their deviations so that the bulk of the relationship can be taken to be due to the straight line. This adds to the value of the correlation coefficients.

Rectilinear relationship was not however true in the case of the other characters—height of plant, emergence or length of peduncle. In these cases the regressions

were studied by equations of the second degree (Figs. 4, 6 and 7). The corresponding coefficients with their standard errors and the proportion of variance eliminated by them are given below :—

TABLE IV.

Coefficients of regression on the yield of the different characters.

Character	Field 2-B		Field 7	
	Rectilinear coefficient	Second degree coefficient	Rectilinear coefficient	Second degree coefficient
Length of peduncle	—1·016±0·291	—0·197±0·037	—0·533±0·249	—0·138±0·035
Per cent. variance	41·5	37·2	23·4	29·7
Value of *P*	<·01	<·01	<·05	<·01
Emergence	—1·974±0·210	—0·144±0·025	—0·948±0·244	—0·144±0·043
Per cent. variance	34·7	10·7	50·1	21·9
Value of *P*	<·01	<·01	<·01	<·01
Height of plant	3·246±0·389	0·249±0·054	0·959±0·290	—0·107±0·056
Per cent. variance	80·4	11·3	42·4	11·3
Value of *P*	<·01	<·01	<·01	0·10 (not significant).

It will be seen that except in one case of height of plant the second degree coefficients are all significant showing that the relationship is more complex than in the previous cases [Fisher, 1932]. An examination of Fig. 4 shows that increased yield goes with increased height right through in Field 2-B. While in Field 7 the relation holds good only up to the mean height beyond which taller plants have not produced bigger earheads. In the case of emergence or length of peduncle (Figs. 6 and 7) the relation is similar in the two fields, and nearly similar for the characters. It is worthy of note that in both the fields changes in emergence or length of peduncle, up to the mean or even a little beyond it, have not produced any appreciable change in the mean weight of head, but plants with a very high emergence or length of peduncle tend to have very poor earheads. It can be inferred that selection for these characters will influence the yield in the manner above indicated, but as these results are affected by the relations of one character with another the independent contribution of each was next investigated.

Partial relationships of yield with the other characters.

The entire series of partial correlation coefficients [Yule, 1923] of yield with other characters at all stages of elimination are given in Appendix, omitting the character, length of peduncle, as not being significant.

An examination of the values shows that the relation of yield to circumference of head remains high at all stages of elimination. The relations of other characters however get profoundly altered when the effect of circumference is eliminated, showing that this is the most intimately connected factor in the yield of the plant. Not only is the magnitude of the final order coefficient small in the other characters, but they vary also considerably from one field to another, except in the length of head ($r_{14\cdot235}$=0·3206 ±·0280 and 0·2655 ±·0360). To judge further their relative contributions, the following regression relationships were next studied.

Yield as a function of the other characters.

If the yield is expressed in terms of the other characters the resulting generalised regression equations are as follows: the values x_1, x_2, ... x_6 all being measured from their means, and the attached coefficients giving the factors by which these have to be multiplied in order to give the change from the mean yield.

Field 2-B

x_1=	10·484 x_2					
=	10·562 x_2	−·3311 x_3				(R_{123}=·9681)*
=	9·606 x_2		+2·924 x_4			(R_{124}=·9702)
=	9·889 x_2	−5·273 x_3	+2·814 x_4			(R_{1234}=·9706)
=	9·618 x_2	−9·418 x_3	+3·395 x_4	+·0918 x_5		(R_{12345}=·9716)
=	9·590 x_2	−6·520 x_3	+3·373 x_4	+·0753 x_5	+·0622 x_6	(R_{123456}=·9724)

Field 7

x_1=	8·997 x_2					
=	6·534 x_2	+52·459 x_3				(R_{123} =·8726)
=	7·890 x_2		+4·307 x_4			(R_{124}=·8654)
=	6·046 x_2	+45·365 x_3	+3·376 x_4			(R_{1234}=·8856)
=	6·107 x_2	+48·468 x_3	+2·894 x_4	+·0682 x_5		(R_{12345}=·8863)
=	5·791 x_2	+47·684 x_3	+3·104 x_4	+·0877 x_5	−·3651 x_6	(R_{123456}= 8902)

Weight of head (1)	Circumference of head (2)	Diameter of peduncle (3)	Length of head (4)	Height of plant (5)	Emergence (6)

1. From an examination of the regression coefficients above noted it is seen that the circumference of head exerts a most pronounced positive influence on the yield in both fields. Considering the standard deviation of this character (σ=2·033

* Fisher's [1932] z test showed that in all cases the multiple correlation coefficients were very significant.

and 1·285 cm.) the effect can be taken to be appreciable, being greater in Field 2-B than in Field 7.

2. The behaviour of diameter of peduncle is different in the two fields (ρ= —5·3 to 45). Although the variability of this character is low (σ_D=0·137 and 0·083), the effect on yield should be considered somewhat comparable to that of circumference of head in Field 7 but not in Field 2-B.

3. The contribution of length of head is similar in the two fields but small (σ_L=0·841 and 0·712). The magnitude of its influence is of a much lower order than the circumference of head.

4. The effect of height of plant is low (ρ=0·068 and 0·092). Even though the character exhibits a large range of variation (σ_H=12·269 and 10·467 cm.) the effect on yield is less than that of length of head and more or less negligible.

5. The influence of emergence (σ_E =3·723 and 3·186 cm.) is different in the two fields but inappreciable. Bearing in mind that in the last two characters the regression is also not linear, their importance is still further minimised.

The extent to which the yield is expressible as a function of the other characters can be understood by the values of the multiple correlation coefficient, R, which are given in brackets at the end of each regression equation. When we remember that the original correlation of yield and circumference of head is so high as 0·9681 and 0·8425, and that the final multiple correlations are only 0·9724 and 0·8902 respectively, the inclusion of the other characters cannot be said to have contributed appreciably to yield. The relative importance of these characters can be understood by the following analysis giving the proportions of the variance eliminated by successive inclusions of the characters in the regression equation :—

TABLE V.

Analysis of variance of different characters.

Character included	Field 2-B				Field 7			
	Freedom	Variance eliminated	Percentage variance eliminated	Residual variance	Freedom	Variance eliminated	Percentage variance eliminated	Residual variance
Circumference of head	455	208579	93·70	14024	298	40532	70·98	16572
Length of head	454	868	·39	13156	297	2244	3·93	14328
Diameter of peduncle	453	222	·10	12934	296	1999	3·50	12329
Height of plant	452	422	·19	12512	295	91	·16	12238
Emergence	451	422	·19	12090	294	302	·54	11930
Mean residual variance				26·8				405·8

It is seen that by far the greater proportion of variance is accounted for by the circumference of head, and that when once this is included the other characters play only a very small part in the determination of yield. Although by comparison with the mean residual variance the variations due to the length of head and diameter of peduncle appear significant, their relative contribution is of a small order. Equation (1) with only circumference of head can thus be taken as a fair indication of the yield. If any more accuracy is required equation (3) comprising length of head can be included, from its consistency of behavior in the two fields. The inclusion of diameter of peduncle is justifiable in Field 7 but not in Field 2-B. The primary factor associated with yield in the plant is however the circumference of head.

Discussion.

Patel and Patel [1929] working on Bombay *jowars* find generally positive correlations of yield with a number of characters within the same types, but annual fluctuations were noted in the figures. In the present studies the correlation between different strains has also been included, in addition to the independent contribution, from multiple regression studies, of the several characters. Many of the relationships are similar in the *pacha jonnas* and the Bombay *jowars*, but exceptions occur. In the Nandyal types for example the contribution of the circumference of head is relatively higher, than the other characters. The effect of length of rachis, which was found to have positive correlations in the Bombay types, could in the present studies be subdivided into a positive contribution from the length of head, and a slightly negative or insignificant relation from the length of peduncle. There are also other differences in the magnitudes of the correlations which are probably associated with varietal causes. The conclusion from the present studies is that the factors contributing to the yield of the plant come from the size characters of the head, especially the circumference of the head. In the case of the other characters although the original correlations are high, the independent contribution is of a small order, being positive for length of head and somewhat variable in others. Since these relations occur in a similar form from type to type, selection on the basis of these characters is justified. As however the yield of the plant (weight of the head) is itself an easily measurable character, the importance of the others are not of much practical application when the heads themselves are available for examination in the laboratory. But in the first year of selection when extensive sorghum surveys are made and material is to be gathered from a great variety of places and conditions, a judicious attention to these characters will include most or nearly all the factors contributing to yield. In this connection the choice of stout and long heads with thick peduncles and an

emergence not much beyond the required limits, is indicated. When we remember that the multiple correlation of yield on inclusion of these characters is so high as 0·9724 and 0·8902, their importance in yield studies is apparent.

Causes of the yield relations.

The above relationships have been determined from correlation studies; but it is well to remember that the correlation coefficient is an index of the proportion of causes common in the genesis of two variables to the total [Bowley,1920] and not the causes themselves. It will therefore be of interest to find the real nature of these basic influences. Some indications in this connection are given below.

The first factor is field variation. It has already been observed that the relationships differ markedly in sign in the two fields as with emergence or diameter of peduncle. In other characters the magnitudes of the correlations were found to differ. For example the more variable Field 2-B (σ_W=22·024 as against 13·720 grms. in Field 7) has produced the greater intensity of correlation in all characters (Table I). This in itself is an indication of environmental effect. Another source of variation was in border influence. The difference between the mean of end-border plants and the centre ones in the two line rows for 34 selections were as follows:—

Table II.

Mean difference between end-border plants and centre ones in the several characters.

Character	Mean difference (border-centre)	S. E. D. M.	Value of *P* (Fisher)
Weight of head	12·258	1·905	<·001
Length of head	0·7030	0·1127	<·001
Circumference of head	1·278	0·1764	<·001
Diameter of peduncle	0·04917	0·00945	<·001
Height of plant	—2·430	2·103	Not significant
Length of peduncle	—0·5250	0·5288	Do.
Emergence	—1·389	0·6039	·03

It will be seen that border plants have given a very pronounced increase in the weight of the head, length of head, circumference of head and diameter of peduncle, and a small, but significant *decrease* in the emergence. The height of plant and length of peduncle are unaffected. As the spacing between selections in this case was so high as six feet, the border effect noted is really the effect of end-border over an already existing line-border influence and should be much greater than was observed. This effect of border is also to make for correlation of yield to the other characters in the manner above indicated.

A third source of variation was noted as the result of manurial treatment. It was found that in the cattle-manure plots and in the plots receiving a combination of cattle manure and artificials the plants were taller and the earheads bigger and earlier than in the 'no manure' controls. Although no measurements were taken, the differences were of a pronounced type and were further noted in all the six replications of the treatments. Here also the effect is to increase the correlation.

It can be concluded from the above that field variation, border effect and manurial responses are all environmental influences, associated with developmental variation, which tend to increase the correlation of yield with other characters from plant to plant. Beside these, genetic forces also operate in the relationships, as the correlations between strain to strain have demonstrated (Table I, Column V). It is variation of the latter kind that is of practical interest to the plant breeder and provides for help in yield studies. The above observations have definitely indicated that genetic variations are likely to be considerably masked by environmental influences. To eliminate such effects, the obvious course for the plant breeder will be to grow his original types in poor fields of low variability without manure, and to give free play to the heritable forces that make for the yield. Selection made under such conditions is likely to be more successful in maintaining yields for the future.

Summary.

1. Judged by their relation to the weight of the head from plant to plant, the characters—circumference of head, diameter of peduncle, length of head, and height of plant—are positively related to yield in the order of importance. The emergence shows a negative relationship.

2. The relation is also true from type to type but in this case the correlations of circumference of head and diameter of peduncle are reduced, while the emergence and length of peduncle show negative effects. The height of the selection and length of head however were found of no significance.

3. The relations of circumference of head, length of head and diameter of peduncle to the yield were found nearly rectilinear, showing a proportional increase or decrease of weight of head along with changes in these characters. The regressions of height of plant, emergence and length of peduncle were more complex.

Plants with very high emergence or length of peduncle tended to produce very small earheads.

4. Judged by their independent contribution to yield the circumference of head showed the greatest relation with it, while the effect of other characters is either

variable from field to field (diameter of peduncle, height of plant or emergence) or of a small order (length of head).

5. The equations of yield expressing it as a function of other characters have also been presented. The circumference of head gives a fairly close indication of yield, and to a smaller extent the length of head.

6. It is shown that part of the relationship noted is the result of developmental variation associated with environmental causes as differential field effect, border influence or manurial response, which are likely to mask the effect of genetic variation between one strain and another. The necessity of selecting original types from uniform, unmanured fields is indicated.

Acknowledgments.

To Mr. K. Gopalakrishna Raju Garu, L. Ag., Deputy Director of Agriculture, Bellary, who directed this enquiry, we are indebted for much facilities and guidance.

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APPENDIX.

Partial correlation coefficients of weight of head (1) with other characters.

Circumference of head (2)			Diameter of peduncle (3)			Length of head (4)			Height of plant (5)			Emergence (6)		
$r12$ etc.	F. 2-B.	F.7	$r13$ etc.	F. 2-B	F. 7	$r14$ etc.	F. 2-B	F. 7	$r15$ etc.	F. 2-B	F. 7	$r16$ etc.	F. 2-B	F. 7
$r12$	·9681	·8425	$r13$	·8694	·7596	$r14$	·7862	·5659	$r15$	·5578	·2936	$r16$	— ·3946	— ·3070
$r12·3$	·8617	·6603	$r13·2$	—·0036	·4216	$r14·2$	·2712	·3680	$r15·2$	·1016	·2627	$r16·2$	·1704	— ·0787
$r12·4$	·9206	·7943	$r13·4$	·6899	·6786	$r14·3$	·4247	·3644	$r15·3$	·1578	·1712	$r16·3$	·0318	— ·2354
$r12·5$	·9538	·8393	$r13·5$	·8090	·7422	$r14·5$	·7807	·5071	$r15·4$	·5440	·0374	$r16·4$	— ·1608	— ·3721
$r12·6$	·9632	·8256	$r13·6$	·8432	·7453	$r14·6$	·7478	·5948	$r15·6$	·5624	·3496	$r16·5$	— ·4026	— ·3602
$r12·34$	·8426	·6418	$r13·24$	—·0585	·3750	$r14·23$	·2761	·3100	$r15·23$	·1044	·2160	$r16·23$	·1727	— ·0898
$r12·35$	·8596	·6680	$r13·25$	—·0229	·397 5	$r14·25$	·3045	·2911	$r15·24$	·1755	·1196	$r16·24$	·1987	— ·1557
$r12·36$	·8659	·6420	$r13·26$	·0261	·4234	$r14·26$	·2876	·3888	$r15·26$	·0727	·2839	$r16·25$	·1556	— ·1357
$r12·45$	·8890	·7973	$r13·45$	·5618	·6781	$r14·35$	·4757	·3274	$r15·34$	·2831	·0233	$r16·34$	·0639	— ·2844
$r12·46$	·9218	·7629	$r13·46$	·6812	·6516	$r14·36$	·4272	·4037	$r15·36$	·1548	·2203	$r16·35$	— ·0018	— ·2585
$r12·56$	·9460	·8164	$r13·56$	·7664	·7200	$r14·56$	·7427	·5199	$r15·46$	·5517	·0953	$r16·45$	— ·1950	— ·3808
$r12·345$	·8349	·6462	$r13·245$	—·1056	·3692	$r14·235$	·3189	·2467	$r15·234$	·1966	·0984	$r16·234$	·1921	— ·1546
$r12·346$	·8480	·6127	$r13·246$	—·0275	.3745	$r14·236$	·2881	·3325	$r15·236$	·0684	·2386	$r16·235$	·1541	— ·1368
$r12·356$	·8633	·6463	$r13·256$	·0094	·3983	$r14·256$	·3122	·3095	$r15·246$	·1461	·1424	$r16·245$	·1731	— ·1735
$r12·456$	·8881	·7660	$r13·456$	·5368	·6487	$r14·356$	·4756	·3526	$r15·346$	·2762	·0676	$r16·345$	·0066	— ·2911
$r12·3456$	·8393	·6191	$r13·245$	—·0725	·3736	$r14·2356$	·3206	·2655	$r15·2346$	·1603	·1211	$r16·2345$	·1549	— ·1697

STUDIES ON THE NATURE OF THE CAUSATIVE AGENT OF THE MOSAIC DISEASE OF TOMATOES.

BY

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(With Plates XLV-XLVIII).

I. DESCRIPTION OF THE DISEASE AND THE METHOD OF THE ISOLATION OF THE CAUSATIVE PRINCIPLE.

In December 1931 many tomato plants in Pusa showed symptoms of a type of mosaic disease. The plants were stunted. The leaves were small, crinkled, deformed and showed distinct patches of yellow markings. Necrosis occurred in very small patches on mature leaves. The disease first made its appearance on the young leaves just emerging ; the leaves that were mature at the time when the disease was just manifesting retained a healthy appearance, but the leaves that were not quite mature showed mosaic markings later on. There were no lesions on the stems, petioles, or fruits.

Many attempts were made to isolate the organisms that might possibly be present in the diseased tissues. For this purpose various tissues were treated with 1 : 1000 mercuric chloride solution for 10 minutes in vacuum, they were then washed with sterile water and crushed. Large amounts of the suspension from the crushed tissues were used for plating on various nutrient agars, namely, tomato extract agar, nutrient agar and marmite mannite agar, but no growth occurred on these plates. Hand sections of the stems and leaves showed no organism in the tissues, and the examination of the juice of the diseased plants also gave a negative result. The repeated failure of isolating any organisms, the appearance and the nature of the symptoms pointed to a virus as the cause of the disease.

Portions of the sterilized and washed diseased tissues were crushed in tomato extract broth and incubated for one week at 30°C. No growth was observed in these tubes and the tissues appeared sterile. The incubated broth was filtered through a sterile filter candle assembled as previously described [Desai, 1932]. This filtrate was used as a stock material of the virus causing the disease.

II. Study of the action of the virus filtrate on some of the soil organisms.

During the course of investigation designed to determine the nature of the infective principle present in the virus of tomato mosaic, Bewley [1931] obtained a bacterial growth showing various degradation forms. At the same time a bacteriophage dissolving these organisms was also isolated from the diseased tissues. The action of the bacteriophage was not very marked because the organisms seemed to have acquired a kind of resistance, owing to their long association with the bacteriophage and showed lysis when the organisms were first freed from the bacteriophage and the purified bacterial culture was used for showing the action of the bacteriophage. As Bewley repeatedly obtained the same type of bacteriophage from various isolations from diseased tissues, he has suggested that the principle causing the mosaic disease of tomatoes is of the bacteriophage type which at first enters the plant with the organisms, it parasitises under natural conditions, and finally becomes adapted to the life within the tissue of the tomato plant and causes the symptoms associated with the mosaic disease.

It was therefore decided to investigate whether the tomato disease virus prepared by us acted as a bacteriophage on some of the organism present on the tomato plants and in soil.

Some tissues of the tomato plants affected with the mosaic disease were crushed without sterilization in nutrient broth and incubated for 16 hours. The cloudy suspension of the tissues was plated to obtain pure cultures of the organisms which were present on the tomato plant and had developed on incubation in the broth. Five organisms, T1 to T5, were selected from the plates for testing the action of virus on them.

Young cultures of the organism were suspended separately in tomato extract broth and 0·2 c.c. of the stock virus filtrate added; the suspensions were then incubated at 30°C. for five days. They were examined every day to see if there was any dissolution of the organisms by the virus acting as a bacteriophage. They were filtered then through a sterile Chamberland L3 filter candle and 0·2 c.c. of this filtrate was again added to the fresh suspensions of the young organisms. Serial transfers were continued, repeating filtration and addition into fresh suspensions of the young organisms at every transfer. In this way 10 serial transfers were made, but the action of the virus bacteriophage was not manifested in the extract. These serial transfers were carried out in the hope that the slight initial virulence which the virus may have for the organisms may get enhanced by serial passages and become demonstrable by lysis of the cultures in the end, but as no sign of lytic action of the virus as a bacteriophage was visible, further serial transfers were discontinued.

Twenty-four representative colonies of the soil organisms were obtained by planting the soil suspensions on Thornton and synthetic agar and transferred to agar slants. The action of the stock virus filtrate by serial passages was tried but showed no dissolution of their suspensions, nor was the growth affected in any other way by the bacteriophagic action of the virus.

The following laboratory stock cultures of different known soil organisms were then selected for studying the action of the virus as a bacteriophage :—

1. *Azotobacter.*
2. *B. radicicola.*
3. *Radiobacter.*
4. *B. megatherium.*
5. *B. subtilis.*
6. *B. albolactis.*
7. *B. mesentericus.*
8. *B. mycoides.*
9. *B. coli.*
10. *B. prodigiosus.*
11. *B. pyocyaneus.*
12. *Micrococcus aurious*

Five serial passages were made as previously described by inoculating 0·2 c.c. of the stock virus filtrate into the fresh suspensions of the respective organisms, and filtering through L3 filter candle after incubating for 5 days.

The dissolution of the organisms by the virus bacteriophage was not perceptible. Some of the broth cultures of the spore-forming organisms have a tendency to clear by flocculation of their growth after a certain time and occasionally it was observed that the suspensions receiving the virus bacteriophage became clear a day or two earlier than the control suspensions. But this could hardly be taken as a definite evidence of the action of the virus as a bacteriophage, because the phenomenon was irregular and did not increase in intensity with successive passages carried out for the enhancement of virulence. Plates were poured to see the plaque formation, but the presence of the bacteriophage could not be established as only pin point colonies developed instead of a uniform spreading growth of the organism both in the control and virus plates.

III. Isolation of organisms from the diseased tissues and their purification.

Fresh attempts were made to see if any organisms developed on special media after prolonged incubation, by planting the sterilized diseased tissue without crushing, directly on the medium.

For this purpose stems of the diseased plants were very thoroughly sterilized in 1 : 1000 mercuric chloride at 37°C. under vacuum for 10 minutes. The tissues were repeatedly washed with sterile water and transferred to a sterile Petri dish, and after cutting them lengthwise with a sterile scalpel they were planted on to the slants of tomato extract agar and incubated. The period allowed for incubation was

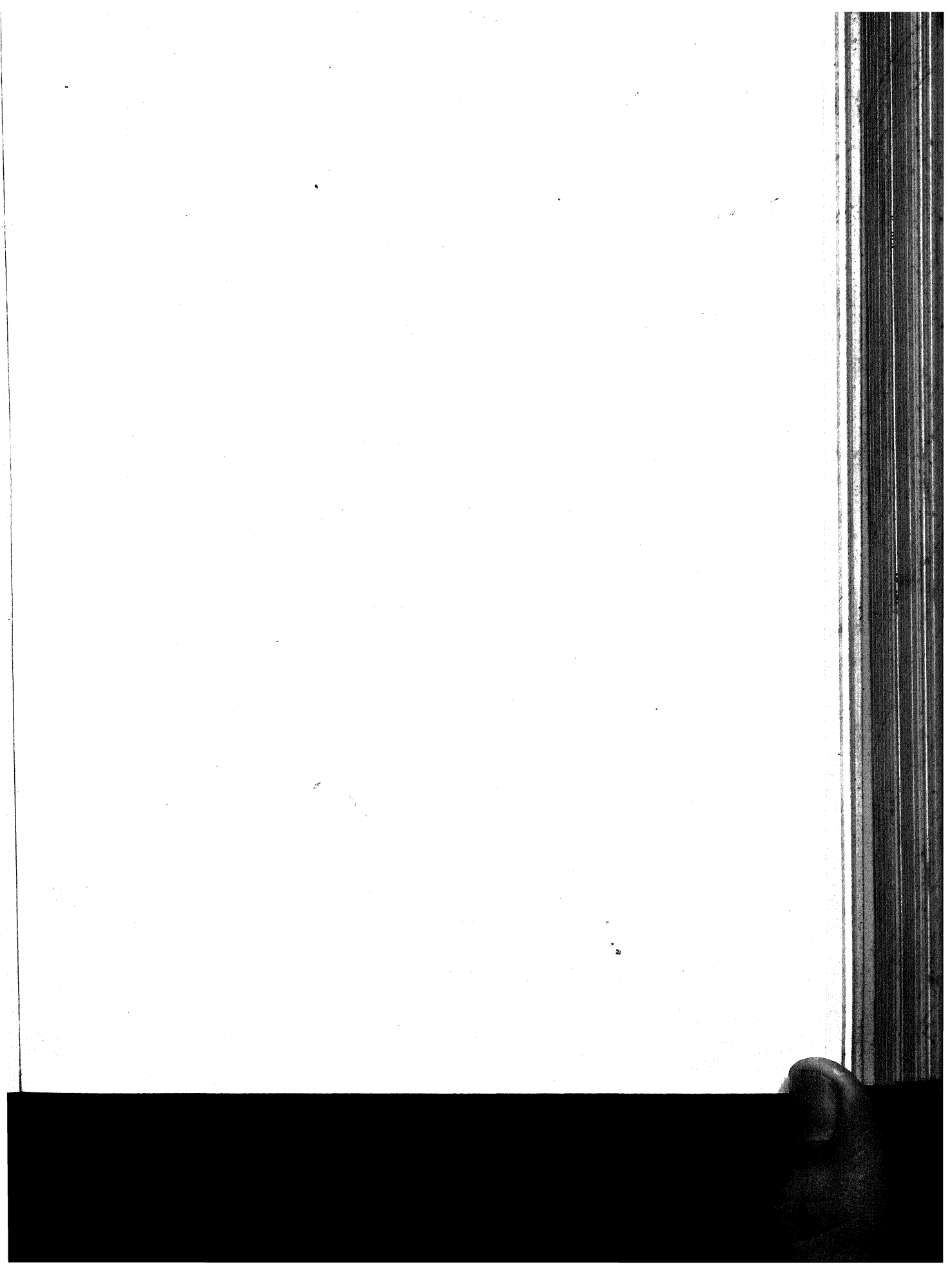


Fig. 1.—Growth surrounding the diseased tissue on tomato extract agar slant (×2).

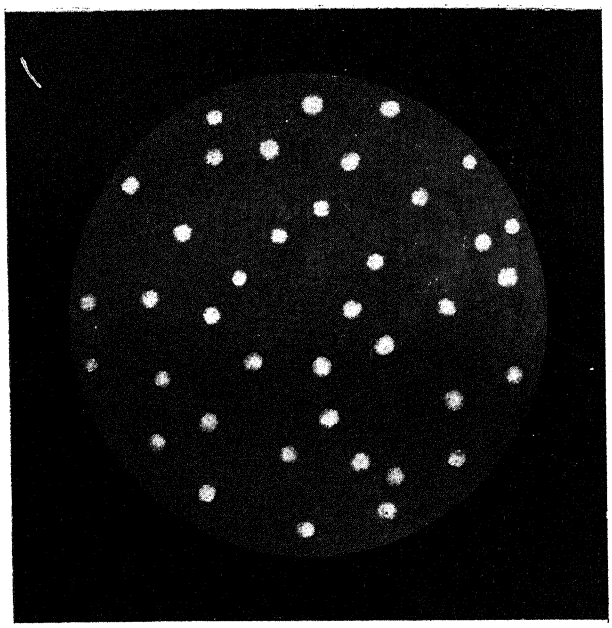


Fig. 2.—Magnified portion of the growth (×12).

much longer than that usually allowed for isolation of bacteria from plants affected with bacterial diseases. The tomato extract agar was prepared as follows :—

Twenty-five grams of green healthy tomato tissues were cut fine with scissors and heated with 1000 c.c. of water for 30 minutes in a sterilizer at 100°C.

The following ingredients were added to the filtered extract :—

0·5	grm.	K_2HPO_4
0·2	,,	$MgSO_4$
1·0	,,	Mannite
0·5	,,	Marmite
15·0	,,	Agar

This mixture was boiled till agar was dissolved and made up to 1000 c.c., filtered, and the reaction was adjusted to pH 7·8 by addition of 1/10 NaOH. For tomato extract broth the same ingredients except agar were used. The medium was finally sterilized at 120°C. for 30 minutes.

The agar slants on which the sterile stems of the diseased tomato plants were placed were sealed and incubated at 30°C. for a long time. Some tubes showed growth after a week and some after a month. The growth was of the same kind in every case (Plate XLV). The characteristic of the growth was that small circular, clear spaces were visible in the growth and that the growth appeared evenly surrounding the tissue. The clear transparent dots were more numerous round the tissue and were little scattered in the growth further away from the tissue. The growth from these tubes on plating showed almost pure cultures of one single type of organisms. Transfers were made from six isolated colonies in the plate on tomato extract agar and on nutrient agar slants. The colonies in the plate as well as the growth on the agar slants showed pellucid dots in their growth. The organisms from these cultures were repeatedly plated but it was found impossible to eliminate the formation of these circular transparent areas.

The appearance of the circular transparent areas was first considered to be due to the association of a bacteriophage with the organisms, as these clear areas resembled plaques formed by bacteriophages in bacterial cultures by dissolution of the organism. Repeated attempts were made to free the organisms from this associated bacteriophage.

They are here described in succession :—

1. Repeated platings and taking inoculum from a margin of an isolated colony yielded no result. The cultures continued to show clear transparent areas as before, the number of such areas remaining approximately constant. Attempts were made to take an inoculum from a giant isolated colony from a part which was well covered with uniform growth and away from any clear area, but on further cultivation this too developed the same kind of growth.

2. As this method for obtaining ultra-pure cultures of the organisms failed, attempts were made to grow the organisms in media rich in sugar as recommended by D'Herrelle [1926] for eliminating bacteriophages from the cultures of contaminated organisms. Media with amounts of different sugars varying from 0·2 to 2 per cent. were made up (mannite, glucose and sucrose were used). The number of clear transparent dots supposed to be representing the bacteriophage particles could not be completely eliminated, though it was noticed that in agar with 2 per cent. glucose and sucrose the number of clear dots were less than that in agar with sugar in lesser concentration. Repeated plating as well as growing in broth of similar composition did not prove of any use.

3. Sugar and other media with different H-ion concentrations were then tried. Tomato extract broth was adjusted to pH values 4·1, 4·9, 6·1, 7·0, 7·6, and solidified with 2 per cent. addition of agar. Cultures of two strains Tb and Tx were plated on the agar of different pH values. It was found that the colonies on agar with a pH value of 7·0 showed the largest number of plaque-like clearings in them. The clearings were large in size in comparison with those in colonies on agar of other pH values. Agar of 7·6 pH value gave next higher number of plaque-like clearings followed closely by agar with pH value 6·1. The number of plaque-like clearings in agar of pH value 4·9 and 4·1 were not so numerous. This was observed to hold good for both the strains, Tb and Tx. Even repeated plating in these acidic agars did not however eliminate the bacteriophage from the organisms. The organisms were then cultivated for four generations in broth with an acid reaction of pH 2·9 and then plated on the acidic agar of pH 4·1. Selected colonies were replated but the transparent clearings still persisted though in smaller numbers.

4. Organisms were next grown in bile extract media for 3 generations being plated on acidic agar between each generation. The colonies which finally developed did not show the transparent plaques prominently, but very minute dots still persisted which on further cultivation assumed normal size, probably due to the adaptation of the culture to the changed circumstances.

5. It was thought that more drastic treatment was necessary and as the organism was a spore-former, it was proposed to heat the culture and plate the spores that remained viable. A suspension containing a few spores was heated on a water-bath to a temperature of 100°C. for 20 minutes. The heated suspension was then plated. No plaque-like clearings could be observed in the few colonies that developed on these plates in the beginning, but as the colony grew large, minute pin-point-like clearings were manifested, and on subculturing this growth, the plaque-like clearings increased in size and numbers and after three transfers the clearings assumed the same appearance as they had before the treatment, the

repetition of this treatment on each successive growth obtained direct from heated spores failed to eliminate the clearings. Similar behaviour of the bacteriophages to persist in spores and resist the high temperature has been observed by Cowes [1931] in case of bacteriophages of spore-formers, *B. anthracis* and *B. magatherium*, and it was believed that in this case too the bacteriophage could not be eliminated. Thus all attempts so far tried have failed to give ultra-pure cultures of the organisms. No progress could be made with the study of bacteriophage supposed to be associated with the organism, as an increase in the virulence of the bacteriophage could not be brought about.

IV. Action of the virus filtrate on the organism found in the diseased tissue.

The action of the stock virus filtrate on six strains of the organisms isolated, as previously described, from the diseased tissues was studied. 0·1 c.c. of the stock virus filtrate was added to the fresh suspensions of all the six strains separately, to see the effect on the growth of the organisms. The tubes receiving the virus-bacteriophage showed slight limpidity, denoting the dissolution or flocculation of the organisms. The tubes showing this clearing were filtered through sterile filter candle and 0·1 c.c. of the filtrate added to the fresh suspensions of the young organisms of all the strains separately. Four serial passages were carried out in this way, but as no difference in the behaviour of the virus bacteriophage could be detected with different strains, one strain Tx 3261 was ultimately used for all further serial transfers and inoculation experiments. The past history of the strain would be of interest in view of the successful inoculation experiments and is given below.

Culture of the organisms isolated from diseased tomato stem :—

1. Plated three times (picking selected colony).
2. Plated twice on 2 per cent. sucrose (picking selected colony).
3. Plated on acidic agar twice (picking selected colony).
4. Cultivated in bile extract broth for three generations with plating on acidic agar between each generation.
5. Spores heated three times and transferred on tomato extract agar several times. At every serial transfer the suspension was made from 24-hour old growth on agar slant.

Twenty serial transfers of the virus-bacteriophage were carried out.

The dissolution of the organisms by the virus-bacteriophage was not very apparent. Nor was the virulence of the virus-bacteriophage found to increase with successive passages as measured by the amount of clearing of the suspensions. It may be considered that as the organisms were associated very closely with a bacterio-

phage which may be the virus principle, in its culture, the virus-bacteriophage could not make its headway to upset the symbiosis and attain enhanced virulence. The filter candle filtrates obtained during the serial transfers showed a secondary growth of the organisms after some time.

The apparent non-dissolution of the broth cultures, the uncontrollable constant plaque-like formation which did not increase on addition of filtrate supposed to contain bacteriophage, and a consistent growth of the same organism in the filter candle filtrate make us hesitate in affirming the virus as a typical bacteriophage, but incline us to the view that the virus is a filterable cyclostage in the life-history of the organisms.

Incontrovertible proofs of the existence in the developmental history of common bacterial species of definitely filterable stages have been given by various recent workers, [Hadley 1931]. The develpment of a pleomorphic and capricious culture when a diseased tissue is placed upon moist agar surface on prolonged incubation lead us to suppose that from the virus stage slowly and steadily visible G. type of growth is being evolved. The plaque-like clearing being the place where culture is in virus form.

The growth of the same organisms from filtrate of L3 to L13 filter candles specially tested for being impervious to becteria confirm us in this supposition.

Appearance of the same type of growth coming out from the diseased tissues in the sealed agar tubes after a long time, say 15 days, would point to the organism as developing from virus to visible stage, as any contamination or bacteria occurring in the tissue would show growth soon after incubation, say in 72 hours at the most, and is likely to be different in different culture tubes.

V. Inoculation experiments and the symptoms of the disease induced.

A number of different organisms have been described as associated with, or as causing one or more virus diseases of plants. Most of these claims have been based on cytological data and have been either disproved or have remained unconfirmed. Few workers who have been able to cultivate organisms on artificial media have not obtained conclusive results in their inoculation experiments [Bewley, 1923; Nelson, 1932].

Uniformly successful inoculation experiments by previous investigators of producing the mosaic disease by inoculating the plants with a culture of any of the organisms found associated with the diseased tissues, have been lacking, and in the light of the accepted views about the agents of the virus diseases any such claim is subject to grave doubts. Since we found one type of the organisms consistently and

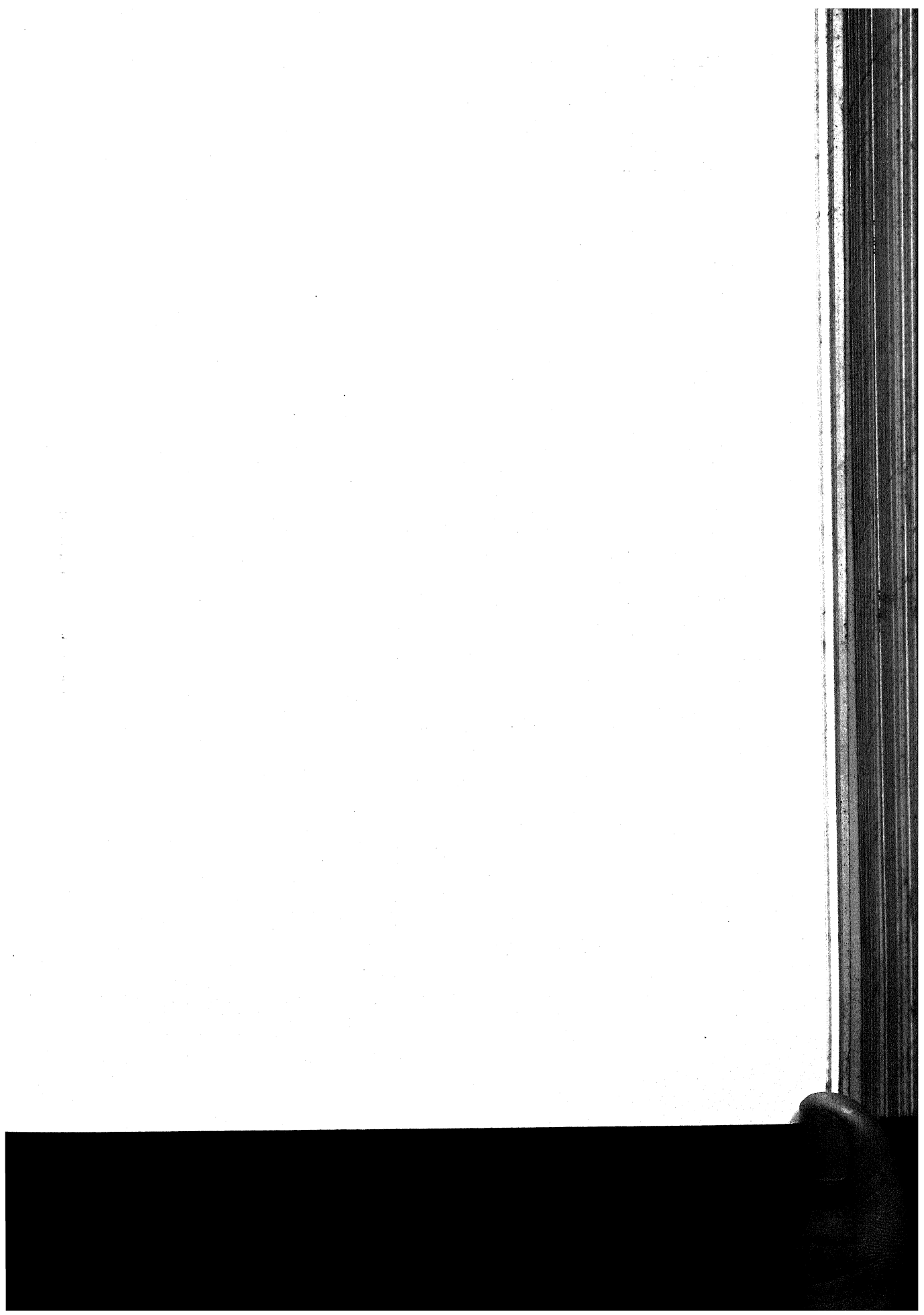
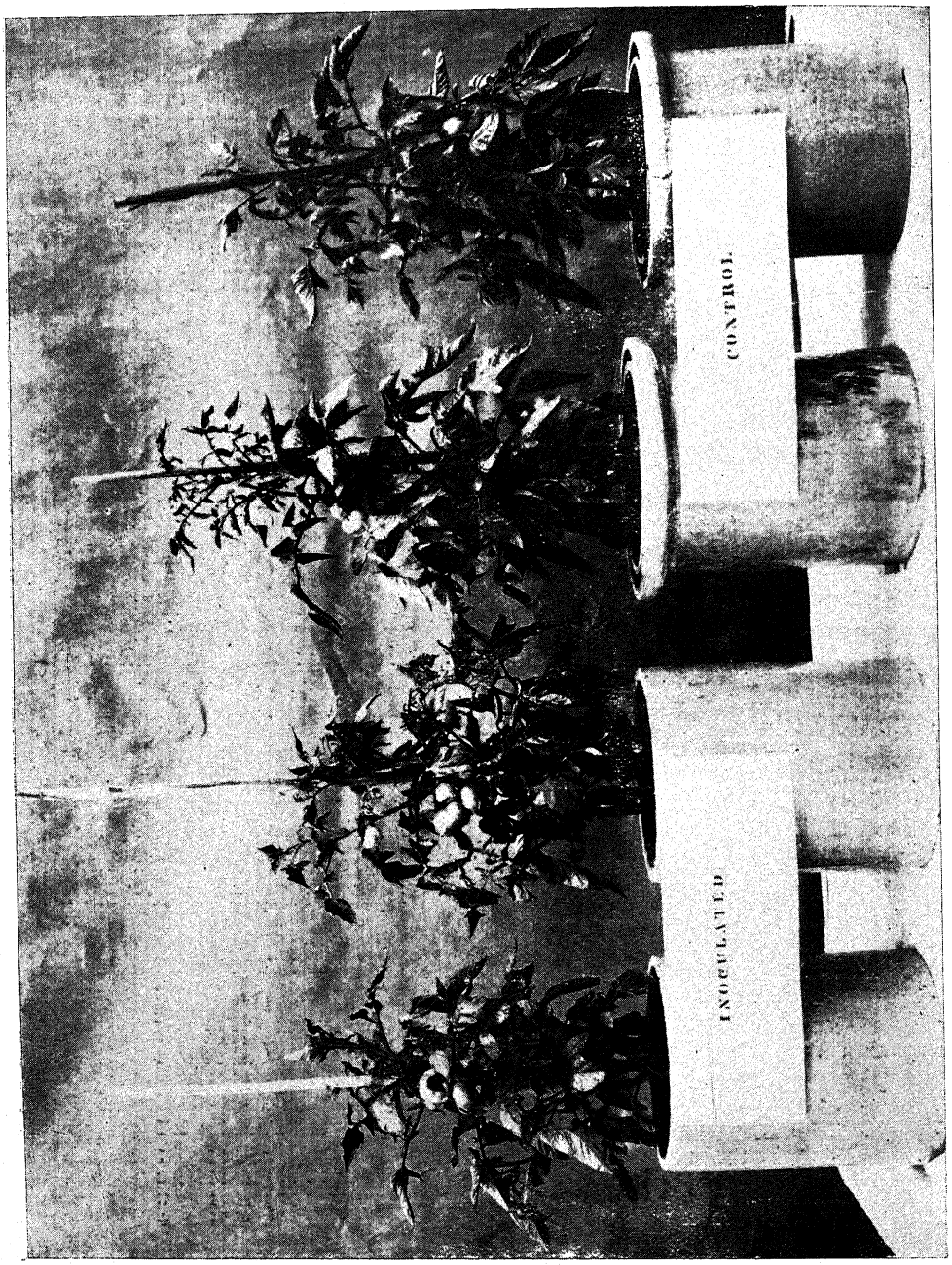


PLATE XLVI.



Plants four weeks after inoculation.

intimately associated with the diseased tissues, it was proposed to inoculate plants with a suspension of the organisms inoculated with an amount of virus diluted to such an extent as to exclude the possibility of producing the disease by the direct action of the diluted virus on the plants and also to secure evidence of the virus multiplying on or with the organisms *in vitro*, simulating the action of a bacteriophage.

The detailed procedure of obtaining the inoculums would clearly show how the extreme dilution of the stock virus filtrate has been arrived at.

Ten c.c. of the suspension of the organisms Tx 3261, whose past cultural history excludes any possibility of the virus being carried in its culture except by being associated like a bacteriophage in spores and growing with the organisms, was inoculated with 0·1 c.c. of the stock virus filtrate from the diseased tomato plants. After incubating the treated suspension for 5 days at 30°C. it was filtered through sterile filter candle; and 0·1 c.c. portion of the filtrate thus obtained was inoculated into a fresh suspension of the 24-hour old culture of the organism Tx 3261. This process of filtration and inoculation in a fresh suspension was repeated serially. It will be seen that with each serial passage the virus of the original inoculum continued to be diluted in the supensions of the successive serial passages to 100 times in geometric progression.

All materials used for making serial passages were scrupulously sterilized.

The first inoculation experiments were conducted with the suspension of the 8th serial transfer. The concentration of the stock virus filtrate in this suspension was 1×10^{-16} of a c.c. This represented a dilution of $1 : 10^{17}$ of the juice of the diseased tissues, (as in the stock virus filtrate the plant juice is diluted ten times) far outside the possibility of the infective dilution of the original inoculum, because the dilutions beyond $1 : 10^{6}$ have failed to produce disease in almost all cases of mosaic virus inoculations, as established by the previous investigators [Henderson Smith, 1928; Allard, 1915; Doolittle, 1920]. The infectiveness of the inoculum of the 8th serial transfer could only be produced by the multiplication of the mosaic virus with or at the expense of the organisms.

The inoculations were made on selected young and healthy plants 15 in. high in glazed pots (Plate XLVI). The inoculum was introduced by scratching the leaves with fine needle pricks dipped in the inoculum. Hands and all material used for inoculation were sterilized just before inoculating. Two plants were inoculated with the suspension of the eighth serial transfers and two other plants with sterile water as control. The signs of the disease began to appear after 10 days in young leaves and shoots of plants inoculated with the suspension. The leaves began to crinkle and small yellow mosaic areas began to appear on them. The stunting of the internodes became evident. These symptoms developed gradually and the difference in the growth of

the control and diseased plants became quite marked. Six weeks after inoculation the difference in height of the experimented plants showed this beyond doubt :—

1. Control	27 in.	25 in.
2. „	23 in.	
3. Inoculated	19 in.	18 in.
4. „	17 in.	

The pots were kept side by side in the open in the experimental field and the control plants remained quite healthy till the end.

Second series of inoculations were carried out with the suspension of the 15th serial transfer. This represented a dilution of 1 : 10^{30} of the original stock virus filtrate inoculum.

A number of young tomato plants were kept under observation for a week, at the end of which period eight healthy plants were selected for the experiment. Four of these plants were inoculated with the suspension of the 15th serial transfer and other four with sterile water as controls. One of the plants inoculated with the suspension 15th serial transfer began to show the usual signs of the disease after seven days and the type of the disease produced was very severe (Plate XLVII). The plant remained very stunted and showed all typical signs of the virus disease. The height of the plant increased only one inch after the inoculation. One other plant of the inoculated series showed distinct signs of the disease a fortnight after the inoculation. The symptoms were quite typical but the disease did not assume a severe form as in the case of the previously described plant.

The third plant of this series also showed the symptoms of the disease, but here again the disease was mild and appeared comparatively late. The fourth plant of this series failed to show any marked sign of the disease though small mosaic-like discoloration could be seen by holding the young leaves against the light. As a distinct sign of the disease, the mosaic colouration without stunting is left out of consideration for the present. All the control plants which were kept side by side were healthy. The heights of the control plants as well as that of inoculated ones before inoculations and six weeks after the inoculation are given below and show the effect of the inoculation quite clearly :

Control.

	Before inoculation.		6 weeks after inoculation.	
1	12-in.	12·5-in.	24-in.	25-in.
2	12-in.		27-in.	
3	13-in.		24-in.	
4	13-in.		25-in.	

Inoculated.

	Before inoculation.		6 weeks after inoculation.	
1	14-in.	14·5-in.	15-in.	17-in.
2	14-in.		17-in.	
3	15-in.		19-in.	
4	15-in.		25-in.	

Fig. 1.—Leaves from inoculated plant, six weeks after inoculation.

Fig. 2.—Leaves from control plant.

The third inoculation experiment was conducted in cages, at a distance from the field where tomatoes were growing and as such the possibility of the disease being borne by insects to the inoculated plants from other diseased plants was reduced to a minimum. The cages used were not absolutely insect-proof but for the purpose of these experiments where the infection could be carried only by a very selective group of insects, there can be no exaggeration in considering the cages insect-proof as the possibility of the disease being caused through insects was excluded. The plants available for this inoculation experiment were not very young as the season favourable to the growth of tomatoes was coming to an end. Four selected plants were put in the insect-proof cages and kept under observation for 10 days so as to exclude the possibility of disease being present in a latent form during the period of incubation after contraction.

Two plants were inoculated with the suspension of the 22nd serial transfer. This represented a dilution of $1 : 10^{44}$ of the original stock virus filtrate inoculum.

Unfortunately a few days after the inoculation a heavy wind storm upset all the cages and these plants were slightly injured and one of the control plants died after this accident. The symptoms of the disease were very late in appearing which may probably be due to the plants being mature and the unfavourable weather conditions for inoculation experiments. Up to three weeks the plants were examined daily for the appearance of the disease but during this time no definite sign of the disease was apparent. The appearance of the disease was noted when the plants were examined nearly six weeks after the inoculation. The inoculated plants had taken the disease and had remained stunted in comparison to the control plant. The leaves were puckered and reduced in size with slight mosaic markings. Under the cage conditions the control plant had grown very tall and thin, the height reached being 48-in. The inoculated plants were 27-in. and 21-in. high only. The stunting was very marked and showed beyond doubt that the inoculation had produced the disease and symptoms other than stunting had not developed prominently. The large increase in the height of the inoculated and control plants may be attributed to the plants receiving an added stimulus for growth in height by insufficient lighting condition of the cages.

Attempts to isolate the organisms from the inoculated plants were made. Besides the leaves which were inoculated, the stems and the petioles of uninoculated leaves were almost invariably found infected with the same organisms, when these tissues of the diseased plants were placed on tomato extract agar after sterilization with 1/1000 mercuric chloride solution and subsequent washing with the sterile water. The leaves of the diseased plants often failed to give growth of the organisms, probably owing to the penetration of mercuric chloride solution into the inner tissues during sterilization with 1/1000 mercuric chloride solution at

37°C. in vacuum. No organisms could be isolated from the stems and petioles of leaves of the control plants after similar treatment, although the incubation was continued on for over two months.

The occurrence of the organism in the diseased tissues without exception and their absence from the healthy tissues leads us to think that the organisms are in some way connected with the production of the disease in tomatoes and that the appearance of the growth of the organisms when the diseased tissues, after sterilization, are put on to agar slants, cannot be put down to any faulty technique.

VI. Morphological characteristics and biological reactions.

The organisms isolated appeared to be pleomorphic and capricious in growth. Sometimes direct slants from the culture failed to show visible growth and sometimes it showed irregular patchy growth. The size of recently isolated cultures varied from 0·1 to 0·3 μ in thickness and 0·5 to 2·5 μ in length. There were many very fine granules in the culture which appeared distinct from well-defined organisms. The granules were deeply stained by ordinary stains, and could not be well differentiated from the stain precipitates. With giemsa and borrel stains the granules appeared very finely stained but in matter of shape could not be definitely said to be different from stain precipitates and could not be distinguished as living entities. Accidentally a preparation was made with Zetnow's flagella stain and it was very interesting to find these small granules having a well-defined flagella. This proved beyond doubt that they were living entities having an ultra-microscopic size (Plate XLVIII).

At first the pleomorphism led us to suspect that we were dealing with a mixed culture but following reasons led us to believe in the unicity of the organisms.

1. Cultures obtained from spores showed the same characteristics.

2. Repeated spore heating and plating revealed only one type of colonies and all show the same type of growth and same pleomorphisms when grown after filtering an old suspension through a filter candle.

3. Growth in different media and at different pH values failed to show heterogeneity of the cultures.

When the recently isolated organisms were cultivated for some time on ordinary agar by repeated transfers, they assumed a constant and well-defined form consisting mainly of quite large rods 0·5 to 0·7 μ in breadth and 1·0 to 4·5 μ in length. The protoplasm was granular and showed presence of small refractile substances. The spore formation was observed to occur very early; even a 24-hour old culture showed nothing but spores.

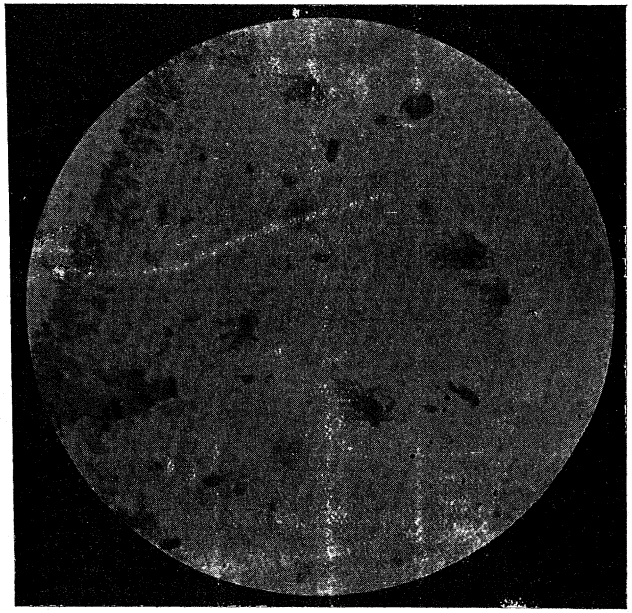


Fig. 1.—Normal size organism with flagella stained.

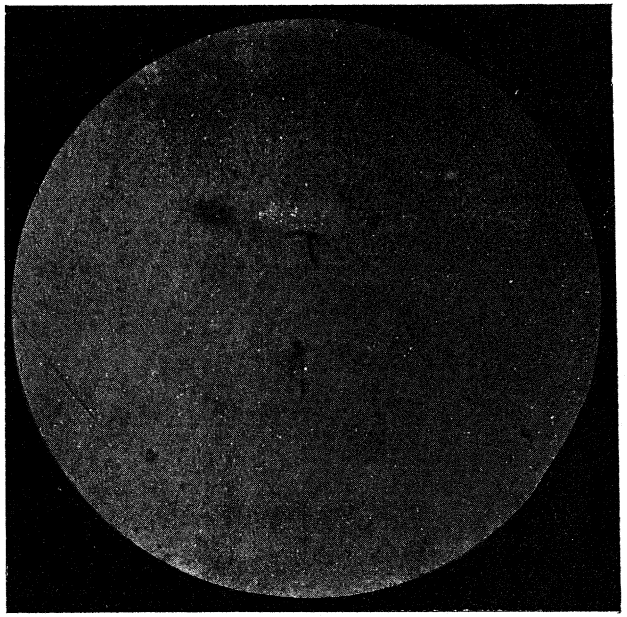


Fig. 2.—Fine granules of organism with flagella stained.

If an old suspension of the stable organisms was filtered through a sterile filter candle, the filtrate on incubation showed a growth of minute granular organisms after 2 days or more. The time taken for the growth to develop varied considerably and sometimes a growth developed in a sealed filtrate tube after 2 months or more. The organisms that developed from the filtrates resembled the organisms just isolated from the diseased tissues in showing pleomorphism and capriciousness with delayed spore-formation.

Spore-formation.—The width of the spores was usually not greater than the organisms from which they arose although a slight bulging was noticeable in some organisms just forming spores. The protoplasm did not remain attached to the spores. The spores were oval in shape, one end being more round than the other and measured $0{\cdot}5\times1{\cdot}2$ μ.

Flagella.—The organisms were actively motile in young cultures; recently isolated cultures which did not form spores readily remained motile for a fortnight or more. The distribution of the flagella was found to be peritrichic.

Gram stain.—The stable forms of the organisms were gram positive but the culture recently isolated from the diseased tissue did not retain the gram stain.

Growth of the organisms on nutrient agar slants was abundant, thick and mealy. The colour of the growth varied from pure white to dirty brownish grey and showed many pellucid dots surrounded by slightly raised rings.

The stab cultures showed growth along the entire line of the stab with much spreading at the top into amæboid colony. The colonies on agar plates were round and raised though at times spreading colonies developed specially when agar surface was moist.

The colonies which were embedded in the agar were stellate and the colonies that developed at the bottom were very thin with a large number of well-defined clear plaques.

Gelatin stabs.—Uniform growth along the entire line of inoculation occurred in gelatin stabs with liquefaction along the growth. The complete liquefaction took place in 48 hours.

Potato.—Thick, greyish white, mealy growth occurred which became yellowish with discoloration of the potato. The growth spread rapidly and appeared moist and mealy.

Litmus milk.—Peptonization began early and progressed rapidly. Slight zone formation was noticed with a yellowish top layer, a deep blue middle layer and a light blue bottom layer. Peptonization continued till all the milk in the tube was converted into a yellowish fluid with a little sediment. Sometimes a slight coagulation was noticed which disappeared with peptonization.

Simple milk tubes showed rapid peptonization and a slight coagulation which soon disappeared. The clear ring of the peptonized milk, which was a very lightly coloured yellow, was observed at the top.

Fermentation of sugars.—Glucose and sucrose were fermented without any gas. The reaction of the glucose medium changed from pH 7·2 to pH 5·6 and that of sucrose from pH 7·2 to pH 5·9. The growth became agglutinated and was precipitated into clusters. Lactose was also fermented without gas.

The growth was not very intense and it was found that the reaction changed from neutral to acidic and again to alkaline. H-ion concentrations were determined and it was found that the reaction of the medium, which was 7·2 pH, changed to 5·8 pH in 48 hours and became 7·9 pH after a week.

ACKNOWLEDGMENTS.

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STUDIES ON *SCHISTOCERCA GREGARIA* FORSK.

THE MICROPYLE IN *SCHISTOCERCA GREGARIA* FORSK. AND SOME OTHER ACRIDIIDÆ.

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(With Plate XLIX.)

Introduction.

While giving a general account of the structure of the eggs of locusts and grasshoppers, Uvarov [1928] states:—

> "At one end of the egg (the upper one when the egg is in the normal position in the ovary of the female or in the egg-pod), there is a small tubercle of a darker colour, with numerous very fine pores in it, through which the spermatozoa enter the egg during fertilization; this group of pores corresponds to the so-called micropyle in the chorion of the eggs of other animals.
>
> "……The end of the egg bearing the micropyle is directed downwards and backwards (in the ovary), and the same position is preserved when the egg is in the egg-mass."

This statement led us to undertake the study of the position and structure of the micropylar apparatus in *Schistocerca gregaria* Forsk. For comparison and confirmation, a general survey of the micropyle in some of the principal sub-families of the Acridiidæ was undertaken.

The following species have been studied:—

Sub-family Tryxalinæ:—

Acrida sp.

Acridella sp.

Sub-family Oedipodinæ:—

Bryodema sp.

Sub-family Pyrgomorphinæ :—

Pœcilocerus pictus F.

Chrotogonus robertsi Kirby.

Sub-family Catantopinæ :—

Schistocerca gregaria Forsk.

Hieroglyphus nigrorepletus Bol.

Hieroglyphodes bilineatus Carl.

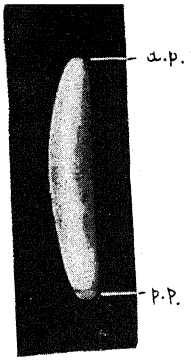
Leuckart [1855] gave a comparative account of the micropyle of insect eggs. Of the Acridiidæ he studied *Oedipoda cœrulescens* (sub-family Oedipodinæ), and *Gomphocerus lineatus*, *G. 2 guttatus* and *G. variabilis* (sub-family Tryxalinæ). Korschelt [1884 and 1887] explained the origin and development of the micropylar canals in the Tettigonidæ. Kunckel de Herculais [1893-1905], in his figure of the egg of *Schistocerca gregaria*, indicated the position of the micropylar canals. Ilienko [1930] has shown a similar structure in his figure of the egg of *Gomphocerus sibiricus* L.

Micropyle of *Schistocerca gregaria* Forsk.

The egg of *Schistocerca gregaria* Forsk. (Plate XLIX, fig. 1) is slightly curved, more or less cylindrical and tapers towards both ends, which are rounded. According to the usually accepted definition [Imms, 1930], the pole of the egg which is cephalad when the egg is in the ovary or in the egg-calyx (and towards which the head of the embryo is finally directed) is the anterior pole. It is this pole which is upper, or nearest the mouth of the burrow, when the egg is in the soil. This pole is narrower than the opposite or posterior pole, which is slightly flattened. The posterior pole is caudad when the egg is in the ovary, or in the egg-calyx, and lower or away from the mouth of the burrow when the egg is in the soil.

It is near the posterior pole that the micropylar apparatus, which consists of a complete ring of canals, is situated* [Uvarov, 1928]. Each micropylar canal is an elongated, funnel-shaped, narrow, chitinous tube running longitudinally towards the posterior pole of the egg. The mouth of the funnel appears on the surface of the egg as an oblique, shallow depression. The canal continues towards the posterior pole as a tube which is oval in cross-section (Plate XLIX, figs. 4 and 5), and becomes narrower as it goes inwards and backwards. It traverses the vitelline membrane and finally opens into the interior of the egg by means of an extremely fine opening. The number of the micropylar canals varies and so does the length of the individual

* For insects in general, Henneguy [1904] and Berlese [1909] state that the micropyle may be situated either at the anterior or the posterior pole of the egg; while Depdolla [1917] says: "..... Am dem vordern Pol ist das Chorion von der Mikropyle, einem einfachen order mehrfachen Porekanale, durchbohrt ....."; and Imms [1930] states that the micropyles "are usually situated at the anterior or cephalic pole of the egg".



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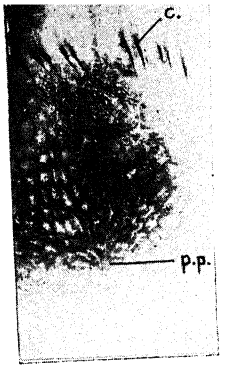

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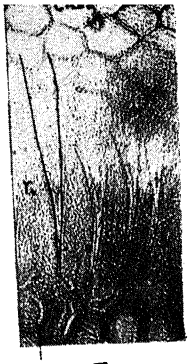

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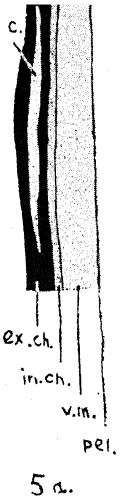

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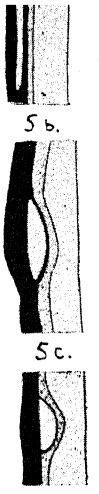

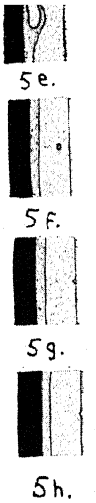

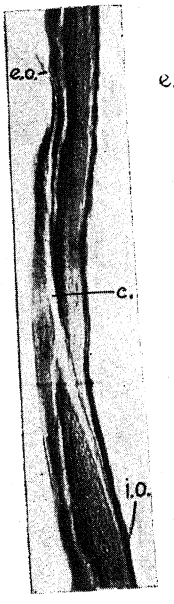

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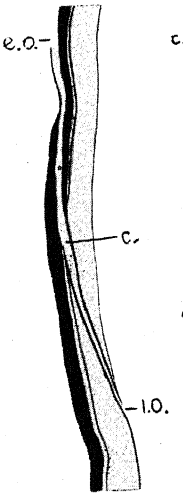

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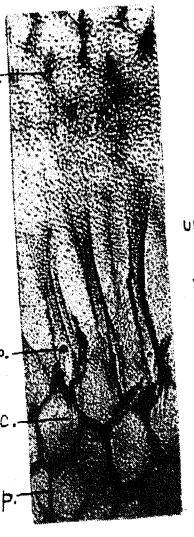

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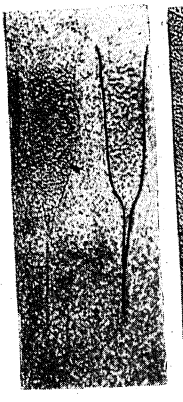
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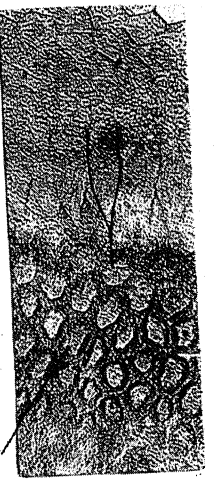

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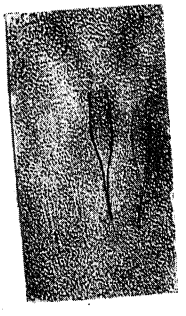
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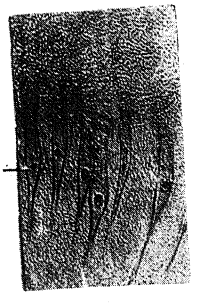

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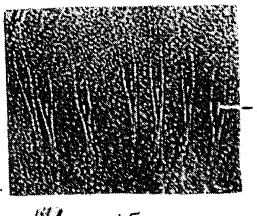

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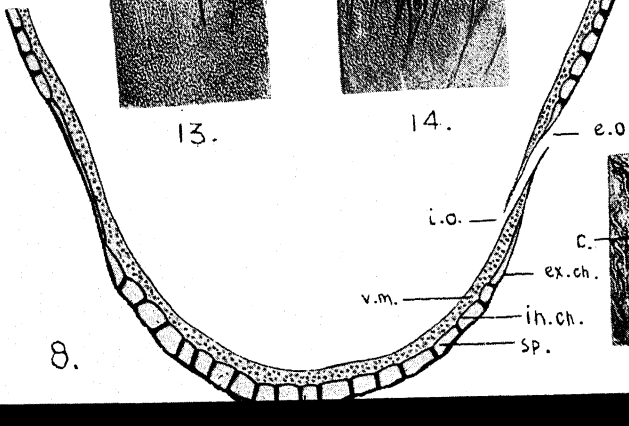

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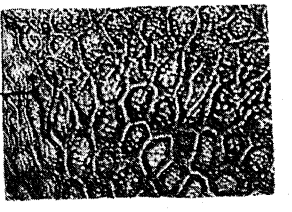

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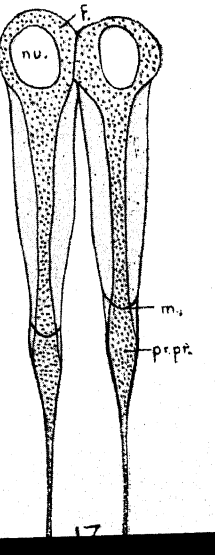

17.

canals (see below). The external openings of the micropylar canals are placed at a distance of about 0·44 mm. from the posterior pole of the egg. The canals lie side by side, but the intercanular distance is not uniform—in some cases the two adjacent canals are so close together that they almost touch each other along their entire length, while in other cases they may be as far apart as 48 μ in the middle of their course. At their exterior end, however, they touch each other. In the region of the micropylar canals the egg is distinctly constricted and the egg shell is more compact. The hexagonal sculpturing which marks the entire surface of the chorion is absent in this region (Plate XLIX, fig. 3). The vitelline membrane is especially thick at the posterior end of the egg.

Eggs obtained from egg-calyx and egg-pod.—Size of eggs (80 readings): Length 4·1-8·0 mm., greatest diameter 0·9-1·6 mm. Number of micropylar canals 44-65. Size of micropylar canals: Length 123-151 μ, width of external opening 7-11 μ. Distance of inner end of canals from the posterior pole about 0·44 mm.

Micropyle in other Acridiidæ.

In all the Acridiidæ examined by us, the fundamental structure of the micropyle is essentially the same as described for *Schistocerca gregaria*. The following is a brief description of the eggs examined.

Sub-family Tryxalinæ.

Acrida sp. (*Plate XLIX, fig. 13*).

Eggs obtained from egg-calyx.—Size of eggs: Length 3·3-3·5 mm., greatest diameter 0·6 mm. Number of micropylar canals 42-53. Size of micropylar canals: Length 113-151 μ, width of external opening 13-23 μ. Distance of inner end of canals from the posterior pole about 0·21 mm.

Acridella sp. (*Plate XLIX, fig. 14*).

Eggs obtained from egg-calyx.—Size of eggs: Length 3·1-3·3 mm., greatest diameter 0·5-0·6 mm. Number of micropylar canals 38-41. Size of micropylar canals: Length 91-132 μ, width of external opening 10-15 μ. Distance of inner end of canals from the posterior pole about 0·16 mm.

SUB-FAMILY OEDIPODINÆ.

Bryodema sp. (*Plate XLIX, figs. 15 and 17*).

Eggs obtained from ovary (*fully ripe*).—Size of eggs: Length 3·5 mm., greatest diameter 0·6-0·7 mm. Number of micropylar canals 52-54. Size of micropylar canals: Length 91-113 μ, width of external opening 11-19 μ. Distance of inner end of canals from the posterior pole about 0·21 mm.

The processes of the follicular epithelium round which the micropylar canals are formed were clearly seen in one case, and are described below.

SUB-FAMILY PYRGOMORPHINÆ.

Pœcilocerus pictus F. (*Plate XLIX, fig. 9*).

Eggs obtained from egg-calyx and egg-pod.—Size of eggs: Length 8·0-8·2 mm., greatest diameter 1·3-1·4 mm. Number of mycropylar canals 63-66. Size of micropylar canals: Length 113-236 μ, width of external opening 15-25 μ. Distance of inner end of canals from the posterior pole about 0·38-0·45 mm.

Chrotogonus robertsi Kirby. (*Plate XLIX, fig. 16*).

Eggs obtained both from egg-calyx and egg-pod.—Size of eggs: Length 3·9-5·0 mm., greatest diameter 0·7-0·9 mm. Number of micropylar canals 49-53. Size of micropylar canals: Length 53-85 μ, width of external opening 10-17 μ. Distance of inner end of canals from the posterior pole about 0·18 mm.

The micropylar canals lie closer to the posterior pole than in the other forms examined. The sculpturing of the chorion is thick-set and is not absent from the micropylar region.

SUB-FAMILY CATANTOPINÆ.

Schistocerca gregaria Forsk.

(Described above.)

Hieroglyphus nigrorepletus Bol. (*Plate XLIX, fig. 12*).

Eggs obtained both from egg-calyx and egg-pod.—Size of eggs: Length 5·4-5·7 mm., greatest diameter 1·4 mm. Number of micropylar canals 59-67. Size of micropylar canals: Length 189-265 μ, width of external opening 23-28 μ. Distance of inner end of canals from the posterior pole about 0·39 mm.

The eggs of this species present certain peculiarities when compared with those of the other Acridiidæ. The micropylar canals lie at some distance from the posterior pole. The egg-wall is very thick, especially at the posterior pole, largely on account of the thickness of the vitelline membrane. The hexagonal sculpturing

of the egg is not strongly marked, but in the hind half of the micropylar region and a little posterior to it there is a band of thick-set hexagons (*th.*), about 0·26 mm. wide, running round the egg and to some extent obscuring the micropylar canals.

Hieroglyphodes bilineatus Carl. (*Plate XLIX, figs. 10 and 11*).

Eggs obtained from egg-pod.—Size of eggs: Length 5·1-5·3 mm., greatest diameter 1·1-1·2 mm. Number of micropylar canals 50-55. Size of micropylar canals: Length 199-246 μ, width of external opening 21-32 μ. Distance of inner end of canals from the posterior pole about 0·31 mm.

The shape of the micropylar canals is somewhat different from those of the other Acridiidæ examined. The canal abruptly narrows down to form the tubular portion; in other Acridiidæ it narrows gradually. Indications of such a shape are seen in *Acrida* and *Bryodema*, but it is most marked in *Hieroglyphodes*.

Origin of the micropylar canals.

Prior to Korschelt's work [*loc. cit.*] no detailed observations regarding the origin of the "compound" micropyle among insects had been made, and the views entertained were largely based on conjectures. Meissner [1854], who believed in the formation of the egg-chorion by a direct transformation of the cells of the follicular epithelium, was of the opinion that the micropyle arose as a result of the absence of the cells of the follicular epithelium at the micropylar end of the egg, and in that region there were apertures (or "micropyles") in the vitelline membrane also. Leuckart [1855] believed that the numerous micropylar canals of *Gomphocerus* did not arise as such but were developed secondarily through the resorption of the chorion and the vitelline membrane. The conjecture of Leydig [1867] that the micropylar canals were secreted round pseudopods, just like the "pore-canals" in the "Hautpanzer" of Arthropods, came nearest the truth. Korschelt [1884 and 1887] proved this to be so by definite and detailed observations on *Meconema varians* (Tettigonidæ), where he showed conclusively that each micropylar canal was laid round a protoplasmic process arising from the cells of the follicular epithelium in that region, and that when the chorion-formation was completed, these processes were withdrawn.

We have been able to confirm this in the Acridiidæ. In a preparation of an ovarian egg of *Bryodema* (Plate XLIX, fig. 17), the epithelial cells (*f*) in the region of the micropylar canals give forth slender protoplasmic processes (*pr. pr.*) filling the cavity of the canals. These processes can be traced into the narrow end of the canal although not right to the tip of the inner end, which, however, they undoubtedly reach. In *Bryodema* each cell in this row has a process.

Summary and conclusions.

The micropylar apparatus in *Schistocerca gregaria* Forsk. and other Acridiidæ consists of a complete ring of minute, funnel-shaped canals arranged near the posterior pole of the egg—the pole directed caudad in the ovary and downwards in the egg-pod. The number of the canals varies. The uniformity exhibited in the structure of the micropylar apparatus, by the species which have been examined so far, suggest it to be the general character of the family Acridiidæ.

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Explanation of lettering.

a. b. Air bubble in the micropylar canal.
a. p. Anterior pole of the egg.
c. Micropylar canal.
c. a. Chorion sculpturing anterior to the micropylar region.
c. p. Chorion sculpturing posterior to the micropylar region.
e. o. External opening of the micropylar canal.
ex. ch. External chorion.
f. Cell of the follicular epithelium.
in. ch. Internal chorion.
i. o. Inner opening of the micropylar canal.
m. Beginning of the actual micropylar canal.
nu. Nucleus.
pel. Pellicle on the inner side of the vitelline membrane.
p. p. Posterior pole of the egg.
pr. pr. Protoplasmic process of the follicular epithelium.
r. Micropylar region.

sp. Air space in the chorion.
th. Specially thickened band of chorion sculpturing.
un. p. Unroofed portion of the micropylar canal – (the obliquely truncated mouth).
v m. Vitelline membrane.
y. Yolk.

Explanation of Plate XLIX.

(*Figs. 1-8.—Schistocerca gregaria* Forsk.)

Fig. 1.—Egg. (The dark constriction near the posterior pole represents the micropylar region). Photograph. ×4½.

Fig. 2.—Portion of the posterior end of egg, showing micropylar canals (*c*). Photograph. ×43.

Fig. 3.—Photo-micrograph of the egg-wall in the micropylar region. (The micropylar canal on the left has been inked). ×170.

Fig. 4.—Photo-micrograph of a portion of the transverse section of the egg across the micropylar region, showing the micropylar canals cut across. (The outer chorion is broken in places). ×190.

Fig. 5. a-h.- Semi-diagrammatic, camera-lucida drawings of the portions of transverse section of the egg-wall across the micropylar region, showing the course of a micropylar canal. ×388.

(*a*) Section close to the outer opening of the micropylar canal
(*h*) Section across the extreme inner end of the micropylar canal which is represented by the depression in the pellicle.

Fig. 6.—Photo-micrograph of a portion of the longitudinal section of the egg, showing the course of a micropylar canal. (The inner opening is not in focus in the photograph, but its approximate position (*i. o.*) is indicated). ×285.

Fig. 7.—Semi-diagrammatic, camera-lucida drawing of the same portion as in Fig. 6. (The thin pellicle on the inner side of the vitelline membrane is not represented). ×229.

Fig. 8.—Semi-diagrammatic, camera-lucida drawing of a longitudinal section of the posterior end of the egg—passing on the right through a micropylar canal and on the left through an intercanular area. (The inner chorion is not differently shaded from the outer, and the pellicle on the inner side of the vitelline membrane is not shown). ×43.

Fig. 9.—Photo-micrograph of the egg-wall of *Pœcilocerus pictus* F., in the micropylar region. ×170.

Fig. 10.—Outline of a micropylar canal of *Hieroglyphodes bilineatus* Carl. ×28.

Fig. 11.—Photo-micrograph of the egg-wall of *Hieroglyphodes bilineatus* Carl. in the micropylar region. The canal on the right has been inked. ×170.

Fig. 12.—Photo-micrograph of the egg-wall of *Hieroglyphus nigrorepletus* Bol. in the micropylar region. The canal in the middle has been inked. Note the specially thickened area (*th.*) of the chorion sculpturing. ×170.

Fig. 13.—Photo-micrograph of the egg-wall of *Acrida sp.* in the micropylar region. The canal in the middle has been inked. ×170.

Fig. 14.—Photo-micrograph of the egg-wall of *Acridella sp.* in the micropylar region. ×170.

Fig. 15.—Photo-micrograph of the egg-wall of *Bryodema sp.* in the micropylar region. ×170.

Fig. 16.—Photo-micrograph of the egg-wall of *Chrotogonus robertsi* Kirby in the micropylar region. The canal on the left has been inked. ×170.

Fig. 17.—Semi-diagrammatic, camera-lucida drawing of two of the micropylar canals of a ripe ovarian egg of *Bryodema sp.* showing their mode of origin around protoplasmic processes (*pr. pr.*) of the basal follicular epithelial cells (*f.*) ×288.

DETERMINATION OF NITROGEN IN SOILS, II.

PROTECTIVE ACTION OF SILICA AS A FACTOR IN THE ESTIMATION OF NITROGEN BY THE KJELDAHL METHOD.

BY

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In the previous communication [Srinivasan, 1932], evidence has been adduced to show that all soils contain certain constituents which interfere with the progress of digestion with hot, concentrated sulphuric acid, and that iron and aluminium oxides are probably concerned in such obstruction. The present paper deals with the chemical nature of the interfering agent, the manner of its functioning and the methods of eliminating difficulties caused by it.

EXPERIMENTAL.

Effects of addition of increasing quantities of iron or aluminium oxide.— Samples (5 grms.) of a specimen of soil (wet cultivated, surface) from the Punjab were treated with 2, 3 and 5 grms. respectively of the two oxides and the digestion conducted by 'dry' and 'wet' methods in the manner outlined in the previous paper. The results have been presented in Table I.

TABLE I.

Mode of digestion	Nitrogen as parts per million (averages)							
	Soil alone (control)	Soil+ 2 grms. of Fe_2O_3	Soil+ 3 grms. of Fe_2O_3	Soil+ 5 grms. of Fe_2O_3	Soil+ 2 grms. of Al_2O_3	Soil+ 3 grms. of Al_2O_3	Soil+ 5 grms. of Al_2O_3	Standard error
'Dry' (official Gunning-Hibbard method)	943	918	894	881	939	887	874	±1·6
'Wet' (after standing overnight with 1:1 acid)	982	980	980	983	978	980	985	±0·6

Considerable difficulty was experienced in conducting the above experiments chiefly owing to the thick and viscous condition of the digesting masses. There

was violent bumping together with the settlement of hard cakes at the bottom. Of the two sets, the 'wet' digested ones were always the more satisfactory, but even they had to be treated with further quantities (20 c. c. in each case) of water so as to ensure smoothness of digestion. The results show, however, that, in spite of the above-mentioned difficulties, the 'wet' treatment led to correct values being obtained in all the cases while the 'dry' one led to decreased efficiency of digestion with increasing additions of iron or aluminium oxide.

The residue left after 'dry' digestion consisted of large lumps made up of brownish particles encased in thick white sheaths of a homogenous substance. Examination of the same showed, however, that it did not contain even traces of iron or aluminium, thus indicating that those elements were not directly responsible for the incompleteness of digestion.

Repetition of the above experiment using smaller quantities of soil and oxides in the same proportions led to smoother digestion. The results (Table II) as also the absence of the added elements from the undigested residue confirmed the previous inference.

TABLE II.

Mode of digestion	Nitrogen in parts per million (averages)							
	Soil (2 grms.) alone (control)	Soil+ Fe_2O_3 (4 : 1)	Soil+ Fe_2O_3 (2 : 1)	Soil+ Fe_2O_3 (1 : 1)	Soil+ Al_2O_3 (4 : 1)	Soil+ Al_2O_3 (2 : 1)	Soil+ Al_2O_3 (1 : 1)	Standard error
'Dry' (official)	943	937	926	920	931	915	920	±1·2
'Wet' (overnight)	982	976	987	976	987	976	987	±0·2

Effect of addition of laterite.—The digestions were next repeated with the addition of laterite, which is naturally rich in both the oxides. To 5-gram lots of the soil, 2 and 5 grms. respectively of laterite were added and the digestions conducted as before (Table III). The results were similar to those obtained in the previous experiments.

TABLE III.

Mode of digestion	Nitrogen in parts per million					
	Soil alone (control)	Laterite alone (control)	Soil+ laterite (2 grms.)	Soil alone (calculated)	Soil+ laterite (5 grms.)	Soil alone (calculated)
'Dry' (official)	943	107	961	918	949	843
'Wet' (overnight)	982	147	1,031	972	1,114	967

Effect of addition of titaniferous mineral.—In view of the previous suggestion of Bal [1925] that titanium compounds are probably responsible for the obstruction of the progress of 'dry' digestion in the case of black cotton soils of the Central Provinces, some experiments were carried out adding increasing quantities of *ilmenite* to 5-gram lots of the specimens of soil used in the previous experiments (Table IV).

TABLE IV.

Method of digestion	Nitrogen as parts per million (averages)				
	Soil alone (control)	Soil + *ilmenite* (0·5 grm.)	Soil + *ilmenite* (1·0 grm.)	Soil + *ilmenite* (2·0 grms.)	Standard error
'Dry' (official)	943	939	936	932	±2·6
'Wet' (overnight)	982	976	974	977	±0·6

It was observed that the progress of the digestions was not appreciably hampered by the presence of *ilmenite*: nor were the values obtained by 'dry' digestion perceptibly affected by the addition of increasing quantities of that mineral. The observations would suggest that the obstructing agent in black cotton soils is not titaniferous magnetite but some other component of those heavy clay soils.

Even after completion of digestion by the 'wet' method, soils treated with *ilmenite* left dark-coloured residues. This was found to be due to the presence of unattacked portions of the added mineral and not to incomplete digestion of the soil.

Effect of addition of different types of organic compounds.

With a view to determining the effect of non-nitrogenous, organic compounds on the efficiency of digestion by the Kjeldahl method, some trials were carried out adding (*a*) starch (1 grm.) which is rapidly destroyed in the soil, (*b*) stearin (0·5 grm.) which is somewhat slowly decomposed, (*c*) rosin (0·5 grm.) which persists for considerable time, and (*d*) paraffin wax (0·5 grm.) which resists decomposition—and conducting the digestion by 'dry' and 'wet' methods as before (Table V).

TABLE V.

Mode of digestion	Nitrogen as parts per million (averages)					
	Soil alone (control)	Soil + starch	Soil + stearin	Soil + rosin	Soil + paraffin	Standard error
'Dry' (official)	941	956	934	939	930	±1·5
'Wet' (overnight)	982	978	980	985	976	±0·3

No particular difficulty was encountered in the digestion of the starch or stearin-treated specimens: those treated with rosin took considerable time for completion while those to which paraffin wax had been added gave the greatest amount of difficulty. In the last case there was considerable swelling and frothing accompanied by violent bumping. The digestion was always smoother in the case of the 'wet'-treated specimens than the 'dry' ones.

In view of the previous observations of Russell [1927] and Pillai and Subrahmanyan [1931] that wax-like compounds and wood-resins are present in certain types of soils, some experiments were carried out treating the soil with petroleum ether or alcohol after addition of 0·5 grm. of paraffin wax, then evaporating out the solvent and finally conducting the digestion in the usual way.

It was observed that treatment with the solvents had greatly improved the smoothness of digestion. The results expressed as parts per million were as follows.—Alcohol-treated, 985; petroleum-ether-treated, 980; water-treated, 976; and untreated ('dry' digested), 941.

Pre-treatment of soil with volatile organic solvents.—Samples (5 grms.) of the soil were treated with 10 c.c. each of alcohol (absolute or 80 per cent.) or petroleum ether and then 'dry' digested in the usual way.

It was observed in all the treated cases that the digestion proceeded as smoothly as in that of the 'wet' one: the average nitrogen values (as parts per million) were also about the same and within range of experimental error.—Alcohol absolute, 981; alcohol (80 per cent.), 985; petroleum ether, 974.

Treatment with acid, alkali or salts.—With a view to determining whether the efficiency of 'wet' digestion could be improved by substituting water with aqueous solutions of different substances, samples (5 grms.) of the soil were treated with 20 c.c. each of hydrochloric acid (1 : 1 aq.), potassium hydroxide (20 per cent. aq.), sodium chloride (one per cent. aq.), and sodium sulphate (one per cent. aq.) respectively. The contents of the flasks were then digested either immediately or after standing overnight. The results (Table VI) showed that the effects of addition of different aqueous solutions were not significantly different from that of water alone.

TABLE VI.

Time of digestion	Nitrogen as parts per million					
	'Dry' digestion (control)	Water ('wet')	HCl 1 : 1	KOH 20 per cent.	NaCl one per cent.	Na_2SO_4 one per cent.
Immediate	944	985	972	976	980	980
After standing overnight	..	988	985	976	981	978

Effect of pre-treatment with oxidizing agents.

(*i*) *Hydrogen peroxide.*—Samples (5 grms.) of the soil were treated with 20 c.c. each of 6 per cent. (Merck's) hydrogen peroxide and then digested in the usual way. The results expressed as parts per million are as follows.—Immediate, 1,045; overnight, 1,036; 'wet' overnight (control), 982.

Repetition of the above experiment with four other soils led to the following results (Table VII):—

TABLE VII.

Treatment	Nitrogen as parts per million (averages)				
	Mandalay rice land surface soil	Dacca highland surface soil	Tellicherry heavy clay surface soil	Nasik dry land surface soil	Standard error
Hydrogen peroxide (20 c.c.)	626	931	1,105	967	±1·8
Water (20 c.c.)	539	836	1,042	905	±2·0
Untreated (control)	472	820	967	859	±3·0

The residues left after 'wet' digestion of three of the abovementioned soils were treated with 20 c.c. each of hydrogen peroxide and redigested after further addition of acid. It was observed, however, that the titre values for the ammonia distilling over were nearly the same in all the cases, *i.e.*, 0·9, 0·95 and 1 c.c. of *N*/30 acid. This led to the suspicion that the preparation of hydrogen peroxide may have contained some nitrogen. The presence of nitrogen was later confirmed by (*a*) digestion of aliquots of hydrogen peroxide and (*b*) subsequent knowledge that the particular preparation had been preserved with barbituric acid.

It may be recorded, however, that although pre-treatment with hydrogen peroxide led to fictitious increase in soil nitrogen, the digestion in such cases proceeded even more smoothly than after water treatment and was generally complete in under one hour.

(*ii*) *Pre-treatment with sodium hypobromite or chromic acid.*—The details of the experiment were the same as those of the previous one with the exception that 10 per cent. hypobromite or 15 per cent. chromic acid was used in place of hydrogen peroxide. The average nitrogen values thus obtained were 980 and 985 parts per million respectively, thus showing that the oxidizing agents did not materially improve the efficiency of digestion.

Effect of size of particle on the efficiency of digestion.

Specimens of soils from Mandalay and Nasik used in one of the previous experiments were ground and divided up into different fractions as follows.—(A), passing 10-mesh sieve but not the 20-mesh one; (B), passing 20 but not 30; (C), passing 30 but not 40; (D), passing 40; and (E), passing 60. Samples (5 grms.) of the different fractions were then 'dry' digested and their nitrogen contents determined in the usual way (Table VIII).

TABLE VIII.

Soil from	'Wet' digestion soil passing 30 mesh (control)	Nitrogen as parts per million (averages)					
		A	B	C	D	E	Standard error
Nasik	908	780	792	850	854	856	±1·4
Mandalay	544	408	422	459	464	466	±1·1

It may be seen from the above that although the efficiency of digestion had appreciably improved as the result of finer division of the soils, the results were, in no case, so satisfactory as those obtained by 'wet' digestion. It would follow, therefore, that, to obtain accurate estimates of soil nitrogen, the 'wet' method should always be followed irrespective of the efficiency of grinding the soil.

After completion of the digestions described in the above experiment, it was noticed that the material left in the flasks consisted mostly of whitish lumps *which were generally several times bigger than the soil particles originally introduced*. It was also observed that the white colour represented only the outer coat while the interior consisted of the brown mass already described. The above observations together with the previous ones of Bal [*loc. cit.*] thus suggested that (*a*) the digestion involved some reaction as the result of which the whitish compound was deposited around aggregates of undigested particles and (*b*) it may be possible to isolate fairly large quantities of the white compound as also the enclosed brown substance, by 'dry' digesting either coarse particles or small lumps of soil.

Some experiments were accordingly conducted, rolling the soil into small pellets and digesting the latter by 'dry' and 'wet' methods. It was noted that although the 'wet' digestion was complete in under 1½ hours, most of the 'dry'-treated pellets remained intact even at the end of 6 hours. When after apparent completion in the latter cases the digested masses were distilled with alkali, any value between 790 and 840 p.p.m. of nitrogen was obtained, while with the 'wet'-treated ones, the correct value was invariably obtained. *The enclosed material was*

fresh, unattacked soil. With a view to collecting a useful amount of the protective compound, a number of pellets were 'dry' digested as in the previous experiment and after removing the unused acid and other water-extractable materials, the residue was repeatedly washed until free from sulphates and then examined under the microscope. It was observed that the outer coat was composed of uniform crystals, thereby suggesting that it was probably a single substance. On splitting the lumps it was noticed that the white layer extended only up to a depth of 1 m.m. below which it was almost exclusively fresh, unattacked soil. Portions near the surface were somewhat grey but the interior remained absolutely unchanged. This observation coming into relief in the case of the bigger lumps, showed clearly that the ineffectiveness of 'dry' digestion was mainly due to a part of the soil not coming into contact with sulphuric acid.

Identification of the protective compound.—Since the residual lumps represented fairly large surfaces and were thus easy to handle, some attempts were made to mechanically separate the white substance by scraping out with a pin. The scrapings proving successful, the material from a number of lumps was thus collected and analysed for its contents. It was observed that the substance was made up exclusively of a highly insoluble form of silica. Iron, aluminium or other metals were not present even in traces, thus confirming the previous observation to that effect.

A similar examination of the insoluble residue left after 'wet' digestion also showed that it was mostly silica. It differed however from that left after dry digestion in that the particles were detached from each other and that no lumps enclosing undigested soil were to be found. The observation thus suggested that whereas in the 'dry' digestion the silica particles held together forming the impenetrable protective layer, they were separated during 'wet' digestion, thus facilitating the acid reacting with all the soil particles.

Further experiments showed that the layer of silica was formed almost immediately after the commencement of the heating, representative specimens of lumps obtained after 'dry' digesting for 15 minutes showing about the same thickness of the protective coat as those left after six hours of digestion. Re-treatment with hot, concentrated sulphuric acid of lumps from which the surface coat of silica had been scraped out showed that the protective layer was again formed to about the same thickness as before and enclosing undigested soil within. On the other hand, a similar lump, when dropped into dilute sulphuric acid and heated, broke up readily into small fragments exposing the previously enclosed soil particles to further action of the acid. In a like manner, soil lumps introduced into water, dilute acid or alkali or salt solution in the form of pellets and then digested with the addition of concentrated sulphuric acid broke up into fragments, thus facilitating the comple-

tion of the reaction. The foregoing observations would suggest that *although both the 'dry' and the pre-treated digestions led to ultimate formation of silica as an insoluble residue, the former involved a heavy deposition of impenetrable silica around the undigested soil particles, while the latter was characterised by the initial dispersion of soil particles prior to commencement of digestion.*

Type of soil and extent of protective action.—Specimens of soils from different parts of India and Ceylon were analysed for their nitrogen contents by 'dry' and 'wet' methods. The descriptions of soils together with the results have been given in the following table :—

TABLE IX.

Soil from	Description	Nitrogen as parts per million (averages)		Difference ('wet'—'dry') per cent. 'wet'=100
		'Dry'	'Wet'	
Kottayam	Red gravel—surface—coconut	978	972	—0·7
Kandy, Ceylon	Laterite—tea	1,437	1,471	+2·3
Jaffna, Ceylon	Sandy	313	322	+2·8
Kottayam	Sandy—wet cultivation	433	442	+2·0
Travancore	Sandy loam—garden soil —surface	140	144	+3·1
Tellicherry	Red sandy loam—surface	679	692	+1·9
S. Bihar	Alluvium—surface	440	470	+6·6
N. Bihar	Calcareous	389	419	+7·4
Tindivanam	Loam—alkaline—surface	175	191	+8·2
Sholapur	Light clay—surface	297	339	+12·4
Nandyal	Red clay—sub-soil	273	313	+12·8
,,	Red clay—surface	248	304	+18·3
Sholapur	Medium black—sub-soil	326	395	+17·5
,,	Heavy clay—black—sub-soil	310	411	+24·4

In the case of the light (sandy or sandy loam) soils, the differences between the results obtained by the two methods are more or less insignificant. It is only in the case of the heavy soils that the inadequacy of the 'dry' method becomes quite marked. This observation together with the previous ones that (*a*) the clay fraction of the black cotton soil led to maximum difference being observed between the 'wet'

and the 'dry' methods [Bal, *loc. cit.*] and (*b*) addition of pure sand had by itself no adverse effect on the value of nitrogen obtained by the 'dry' method [Srinivasan, *loc. cit.*] lead to the inference that the protective coat of silica is derived from the complex alumino-silicates which constitute the major part of the finer fractions of the soil.

Mechanism of formation of the protective layer.—With a view to determining whether the formation of the protective coat of silica could be artificially demonstrated in the case of different silicates, some experiments were carried out coating lumps of soil, chips of wood or pieces of metal with sodium or aluminium silicate and subjecting them to the action of concentrated sulphuric acid as in the usual 'dry' digestion. It was observed that although there was not much perceptible action in the cold, the silicates reacted readily with hot acid forming hard, white encrustations which were subsequently identified as being made up of silica. In the case of sodium silicate, the reaction was accompanied by swelling and the escape of bubbles of water vapour; in that of aluminium silicate, it was characterised by the separation of the corresponding sulphate in acid solution together with the release of water which escaped on heating. Owing however to the punctures formed by the escaping vapours, the enclosed materials were not fully protected from the action of the acid, but there were always little lumps which had been so well enclosed that the acid could not penetrate them—an observation corresponding to that made in the course of the ordinary 'dry' digestion of soils.

The above experiments were then repeated, coating pieces of wood or copper with clay paste, and it was noted that similar layers of silica were formed protecting the major portion of the enclosed materials from further action of the acid. The swelling was not so marked as in the previous cases, but potassium, iron and aluminium were detected in the acid solution thus indicating that the chemical nature of the reaction was similar to that observed in the case of the simpler silicates.

The nature of the reaction between sulphuric acid and different silicates after pre-treatment with water or aqueous solutions of acids, alkalis or salts was next studied. Pieces of wood or metal were coated with sodium or aluminium silicate or clay from different sources and then treated with the different fluids followed by concentrated sulphuric acid and the mixtures digested in the usual way.

It was observed in all the cases that within a very short time after the commencement of the heating, the outer layers of silicate or clay were burst into and the enclosed materials rapidly exposed to the action of dilute sulphuric acid. With further heating and the consequent concentration of the acid, lumps of silica began to be formed, but as they were detached from the mass of the digesting material, they offered no resistance to the progress of the reaction. Analyses of the acid

extracts at different stages showed that the corresponding sulphates had been formed in all the cases even before the formation of lumps of silica. This would indicate that the reaction proceeded in two stages, one, the formation of silicic acid and the corresponding sulphate, and the other, dehydration of silicic acid and the attendant precipitation of silica. The former was the result of reaction beween dilute sulphuric acid and the silicates, while the latter occurred only after the concentration of the acid. The two stages could then be represented by the following equations.

$$X_2SiO_3 + H_2SO_4 \text{ (dilute, cold or hot)} \longrightarrow \underset{\text{(in suspension)}}{H_2SiO_3} + \underset{\text{(in solution)}}{X_2SO_4} \quad \ldots\ldots(1)$$

$$H_2SiO_3 — H_2O \xrightarrow{\text{hot, conc. } H_2SO_4} \underset{\text{(precipitate)}}{SiO_2} \quad \ldots\ldots(2)$$

when X represents a monovalent basic radicle. The reaction would follow a similar course even in the case of the complex aluminosilicates, like those present in the soil.

As distinct from the above, the reaction between hot, concentrated sulphuric acid and different silicates would appear to take place at one step, the formation of silicic acid and its subsequent dehydration being almost concurrent.

$$X_2SiO_3 + H_2SO_4 \text{ (hot, conc.)} \longrightarrow \underset{\text{(in acid solution)}}{X_2SO_4} + \underset{\text{(protective layer)}}{SiO_2\downarrow} + \underset{\text{(vapour)}}{H_2O\uparrow}$$

Discussion.

Although it is generally known that silica is one of the insoluble residues left after acid digestion of soils, the importance of the part it plays in determining the efficiency of the reactions leading to the formation of ammonium sulphate has not so far been understood. The foregoing observations show that silica is not formed in the earlier stages of 'wet' digestion, so that the penetration of the entire mass of soil by dilute acid is greatly facilitated: all the particles of soil are thereby acted upon and the digestion thus proceeds to completion. On the other hand, silica is one of the very first products of 'dry' digestion, forming as it does, a thick deposit around undigested soil particles; it forms an impenetrable barrier protecting the latter from further action of the acid. The reaction thus becomes incomplete, yielding reduced estimates of total nitrogen.

The residue left after 'dry' digestion is not acted upon by concentrated sulphuric acid, but is readily penetrated by the dilute acid or any one of the other fluids experimented with. On heating the residual mass left after dry digestion

with dilute acid, the lumps break up exposing their entire contents to further action of the acid. The digestion then proceeds to completion, thus facilitating the estimation of the entire amount of nitrogen left in the residue.

The foregoing observations show clearly that however carefully the digestion be conducted, the official 'dry' method will invariably lead to incomplete reaction between soil and concentrated sulphuric acid : reduced values for total nitrogen will thus result, the error in the estimate increasing with the proportion of clay in the soil. The 'wet' method adopted in the present investigation is a distinct improvement on the hitherto official method, but further work will be needed to standardize the conditions so as to secure the most satisfactory results with all types of soils. The need for quicker and smoother digestion is also indicated and the observations made in the case of specimens treated with hydrogen peroxide suggest that further improvement along this line is possible. No method has so far been devised for obtaining absolute values for the total nitrogen present in a soil, so that although the wet method is a definite improvement on the dry one, the extent of its approach to the real amount of nitrogen in a soil is still obscure.

The observations with regard to the origin and mechanism of protective action exhibited by silica suggest that similar difficulties may be encountered wherever silicious materials are present. They also indicate that the 'wet' method can, with advantage, be extended to the estimation of nitrogen in rocks, minerals and mineral earths which resist digestion by the 'dry' method, to various organic manures which contain soil and other silicious materials, to plant materials (like paddy husk) which are rich in silicious constituents and such other estimations.

Summary.

1. Addition of increasing quantities of iron or aluminium oxide or laterite to the soil renders the progress of 'dry' digestion somewhat difficult, but those substances are not directly responsible for the incompleteness of reaction observed in such cases. Smooth digestion, as also accurate values, can be obtained by adopting the 'wet' method under such conditions.

2. The presence of titaniferous mineral or different types of organic compounds do not appreciably affect the progress of reaction between soil and concentrated sulphuric acid.

3. Pre-treatment with volatile organic solvents or aqueous solutions of acids, bases or salts leads to higher values being obtained than by the official 'dry' method, but the results do not show any improvement on similar treatment with water alone.

4. Addition of hydrogen peroxide greatly hastens the progress of digestion, but allowance should be made for the nitrogen present in commercial preparations of the

peroxide itself. Pre-treatment with other oxidizing agents does not lead to improvement on the values obtained by the 'wet' method.

5. Grinding the soil to a fine state of division leads to higher nitrogen values being obtained by the 'dry' method, but the figures thus obtained are always lower than those secured by 'wet' digestion.

6. The residue left after 'dry' digestion was found to consist of undigested soil surrounded by thick coats of silica. The latter being impenetrable to concentrated sulphuric acid, the enclosed soil particles were thus found to be effectively protected against the action of the acid.

7. The protective action of silica was more marked in the cases of heavy soils than in those of light ones.

8. Study of the mechanism of formation of the protective layer showed that (*a*) silica was the immediate product of reaction between hot, concentrated sulphuric acid and the alumino-silicates present in the soil, so that the protective coat was generally formed before the acid had wetted all the soil particles, (*b*) when soil was heated with dilute sulphuric acid as in 'wet' digestion there was no formation of silica until all the water had been driven out so that there was always sufficient time for the acid to react with all the soil particles and (*c*) the layer of silica formed under conditions described in (*a*) though resistant to the action of concentrated sulphuric acid was readily penetrated by various other fluids including dilute sulphuric acid which further caused the lumps to break up thereby exposing the enclosed soil particles to the action of the acid and thus facilitating completion of digestion.

9. The nature of further work leading to the standardization of a new method of estimating nitrogen in soils, manures, plant products and other materials containing silicious constituents is indicated.

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THE EFFECT OF TEMPERATURE ON THE BREAKAGE OF RICE DURING MILLING.

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During the course of a series of laboratory milling tests on rice it was observed that the percentage of brokens tended to increase from early morning to about 2 p. m. and then to decrease. The following experiments were done to discover if this systematic variation was related to the temperature.

The milling apparatus used has been described by Hayes [1932]. The procedure in all cases was to take a number of samples of paddy, hull in the *kyeik* (rotary huller), winnow, take the temperature of the *iondi* (unpolished brown rice) at the time it was placed in the *moung* (treadle pounding machine) and polish with 500 blows. After removing the bran and thoroughly mixing, samples were separated into broken and unbroken and the percentage of brokens calculated from the latter because small quantities of chips are lost from the brokens but loss of whole grains is rare.

Three pure lines of rice have been tested, namely, Theikpan Ngasein 2104, Theikpan Taungdeikpan and Theikpan Shweat. The first is a bold-grained type with a little abdominal white, usually along the edge. It belongs to Beale's [1927] Class C. The second is a small fine-grained type, glassy, and fits nearly into Beale's Class B but is rather small. The last is also a small Class B grain, slightly broader than Taungdeikpan and it frequently has a small amount of abdominal white along the edge.

The figures obtained for Ngasein 2104 are given in Table I. It was found that very small changes in the setting of the pestles of the *moung* caused large differences in breakage and the machine being mostly wooden, differences in

humidity appeared to cause irregularities. It is therefore not possible to combine the figures for the five tests but each must be dealt with separately.

TABLE I.

Temperature and per cent. breakage for Theikpan Ngasein 2104.

First test		Second test		Third test		Fourth test		Fifth test	
Londi temperature °C.	Per cent. breakage	Londi temperature °C.	Per cent. breakage	Londi temperature °C.	Per cent. breakage	Londi temperature °C.	Per cent. breakage	Londi temperature °C.	Per cent. breakage
32·5	50·3	34·6	52·7	29·6	52·95	29·0	51·4	29·6	55·1
33·2	52·6	35·6	52·5	30·0	52·87	29·5	53·2	30·0	55·3
34·4	53·2	35·2	56·6	30·3	54·10	29·6	54·2	30·0	55·3
35·2	55·2	38·0	54·6	30·2	54·70	30·2	54·5	30·4	56·3
35·7	55·9	38·4	55·8	31·8	55·10	30·7	54·8	30·6	56·8
36·7	58·4	38·2	58·0	32·6	55·60	32·2	54·9	30·6	56·1
36·6	54·1	38·5	58·0	33·6	56·84	32·1	55·0	30·9	56·7
38·0	58·4	32·9	52·7	33·8	56·70	32·8	55·1	..	..
36·9	54·4	33·6	54·8	33·4	56·27	32·2	55·0	..	..
38·4	57·5	34·2	55·0	33·7	57·20	33·0	55·9	..	..
37·5	54·2	34·4	56·0	..	..	33·7	57·2	..	..
39·0	58·9	35·8	57·2	..	..	33·8	57·1	..	..
..	..	36·0	57·7	..	..	34·0	58·0	..	..
..	..	35·8	56·7	..	..	33·8	57·6	..	..

In Table II are given the correlation coefficients for temperature of *londi* and breakage per cent.

TABLE II.

Correlations between londi *temperature and breakage for Ngasein 2104.*

	r	z
First test	0·8347	1·2033
Second „	0·5359	0·5984
Third „	0·9565	1·9033
Fourth „	0·9164	1·5663
Fifth „	0·9256	1·6267
Combined	0·8609	1·2969

With the exception of the second test all the correlations are very high. It is suspected that during the second test one or more of the pestles was out of adjustment.

Omitting the second test the weighted estimate of z is 1·5448 corresponding to $r = ·9282$. There is therefore no doubt that breakage increases as the temperature of the rice before polishing rises.

Estimates of the regression line from such small samples are uncertain but the regression coefficients of breakage on temperature for the second test = ·5655 and for the fourth test = ·9273. It appears that for each degree centigrade rise in the temperature of the unpolished rice the breakage increases not less than ·5 per cent. At the time the paddy was hulled samples were taken for moisture tests as it was thought formerly that changes in moisture content largely affected breakage. Within each of the above tests the variation in moisture per cent. was small and the correlations with breakage all small and insignificant. They are given in Table III.

TABLE III.

Correlations between moisture per cent. of paddy and breakage.

	r	Approximate *P*
First test	+·0665	0·84
Second ,,	—·0468	0·88
Third ,,	+·0187	0·96
Fourth ,,	+·2514	0·40
Fifth ,,	+·2557	0·50

Two tests with Taungdeikpan have been done and the figures are given in Table IV.

TABLE IV.

Temperature and per cent. breakage for Theikpan Taungdeikpan.

First test		Second test	
Londi temperature °C.	Per cent. breakage	*Londi* temperature °C.	Per cent. breakage
31·1	13·6	33·9	27·3
31·9	13·2	34·6	29·5
32·6	12·5	34·5	26·8
33·8	15·3	36·9	29·5
33·8	14·1	37·1	30·5
35·0	19·1	37·3	29·7
34·8	22·2	28·8	25·6
35·3	31·3	29·6	25·4
35·4	22·8	30·7	24·6
36·4	31·9	31·2	23·6
35·9	26·7	31·6	26·1
37·8	34·0	32·2	24·9
		33·4	27·0
		33·6	25·6
		33·6	26·4
		33·9	27·2

In Table V the correlations are given. In this case also they are high.

TABLE V.

Correlation Coefficients for breakage and londi *temperature for Theikpan Taungdeikpan.*

	r	z	$n-3$	$z(n-3)$
First sample	0·8912	1·4276	9	12·8484
Second sample	0·8482	1·2496	13	16·2448
Combined	0·8674	1·3224	22	28·0932

As in the case of Ngasein the correlations between moisture percentage in the paddy and breakage were small and insignificant, namely —·3746 and +·0341 for the first and second tests respectively.

The regression coefficients for Taungdeikpan were 3·69 and 0·636 respectively, a very wide discrepancy, but it is fairly certain that whatever the true value it is of sufficient magnitude to be of consequence. It would probably be safe to assume that each degree rise in temperature is accompanied by at least 0·5 per cent. increase in breakage. It may be considerably more but sufficient data are lacking.

In the case of Theikpan Shweat only a single test has been done and the figures are not so consistent as desirable owing to maladjustment of the *moung* and the small range of temperature. They are given in Table VI.

TABLE VI.

Temperature of londi *and per cent. breakage for Theikpan Shweat.*

Londi temperature °C.	Per cent. breakage
29·1	52·7
30·8	53·2
32·4	52·4
32·0	53·7
32·0	55·0
32·2	55·5
32·8	56·9
32·4	55·4
28·4	52·8
29·0	53·5
29·4	54·6
29·3	53·9
29·2	53·5
29·7	53·1
30·8	53·6
30·8	54·9

A correlation of ·5674 between *londi* temperature and breakage per cent. was obtained, lower than for the other two varieties but nevertheless reliable (P about ·02). The regression coefficient of breakage on temperature was 0·56.

Although the data available do not permit any certain conclusions regarding the regression of temperature on breakage, they demonstrate the close relationship between these variables and that the control of the temperature during polishing is of practical importance to millers and may be a source of serious error in laboratory milling tests.

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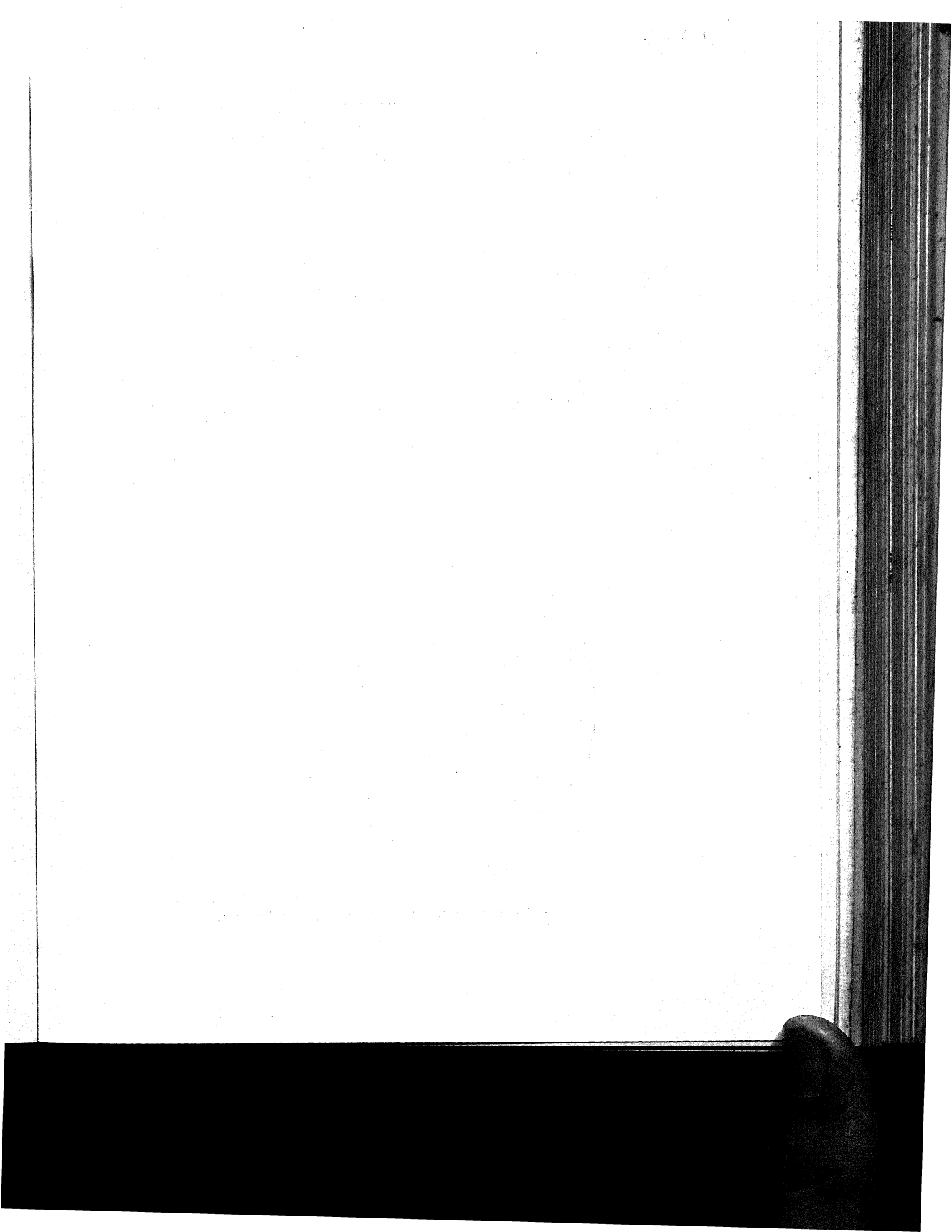

PLATE L.

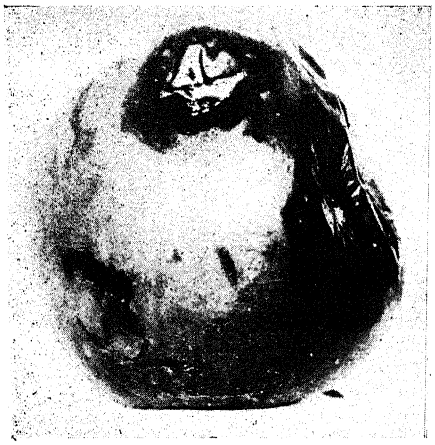

Fig. 1.—The soft rot of apple.

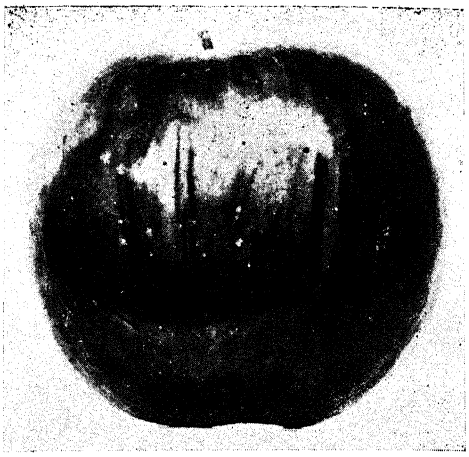

Fig. 2.—An Amrhi apple after twentieth day of inoculation with *A. niger*.

Fig. 3.—Purple brown masses of spore-heads in irregular zones.

A SOFT ROT OF APPLE.

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(With Plate L and three text-figures).

I. Introduction.

A very large proportion of the apples in the markets in the United Provinces are usually found to be damaged by a kind of soft rot. This rot may be only a small circular spot a few millimeters wide or the whole apple may be converted into a pulpy mass. According to the extent of the rot the market value of the fruit is reduced or it may become totally unfit for consumption and unsaleable. The dealers are thus put to a great deal of loss every year. All the varieties are affected, but the sour ones are particularly susceptible.

In the earlier stages the decay is characterized by a small water-soaked area surrounding a bruise or an insect puncture. It soon increases in size, assuming a pinkish brown colour and becomes quite soft and sunken. The browning is more prominent on the green or yellow varieties (Plate L, fig. 1). Within a few days the rot spreads to the whole of the apple, changing it into a soft pulpy mass. The dark brown sunken patch is surrounded by a lighter area which sometime appears as an irregular zone. As the patch becomes progressively softer it sinks further, and the skin, which remains unaffected and intact over the patch, is thrown into wrinkles and appears more shiny and moist. No fructification of any kind is noticed on the surface of the rotted apple. Huber [1930] has described similar decay of apples as his form No. 5, which he found to be caused by *Aspergillus niger*.

II. Method.

The soft flesh from the interior of the rotted portion showed, under the microscope, a network of fungal hyphæ permeating the mass, while the host cells floated loose in the mount. In order to isolate the fungus a number of apples in varying stages of rotting was selected. They were dipped in mercuric chloride (·01 per cent. solution) for half an hour and then repeatedly washed with sterile water. A slit was made near the periphery of the rotted area with a flamed knife,

the skin was peeled off and with a sterile needle a small portion of the flesh was quickly transferred to a sterile Petri dish containing 7·5 per cent. rice agar medium. A large number of such transfers was made in separate dishes. The dishes were kept at room temperature (average mean. 72°F.). Within three days there was, in all the Petri dishes, a good fungus growth spreading on the surface and partially submerged just below the surface of the medium. With a few exceptions every such isolation gave the same type of fungus colony.

Transfers from the extreme edges of these colonies were made in Czapek solution agar medium in Petri dishes. In this medium also there were on the third day, submerged colonies similar to those developing on rice agar medium, about 2 cm. in diameter. In the middle of the colonies appeared purple brown masses of spore-heads arranged in irregular circular zones (Plate I, fig. 3). The colour of the medium remained unchanged, and the colony appeared white and felted from below.

In its morphological characters, described below, this fungus agreed with that of *Aspergillus niger* Van Tieghem. The fungus isolated from this soft rot of apples may thus be similar to Huber's [1930] Form No. 5.

III. Morphological characters of the fungus, growing in Czapek solution agar medium.

The head.—The heads in mass appeared brown with a purple hue; singly they were light brown; globose 75-100μ in diameter,* slightly rimose at the periphery; viewed from the top, the mature heads appeared stellate with a number of irregular stella or arms, each made up of a bunch of chains of spores. The vesicle was globose, rather thick walled, 60-70μ in diameter,* faintly coloured. The sterigmata were uniform in one series, closely radiating from the entire surface, non-septate, without proliferation, 7μ long 3μ broad (Fig. 1).

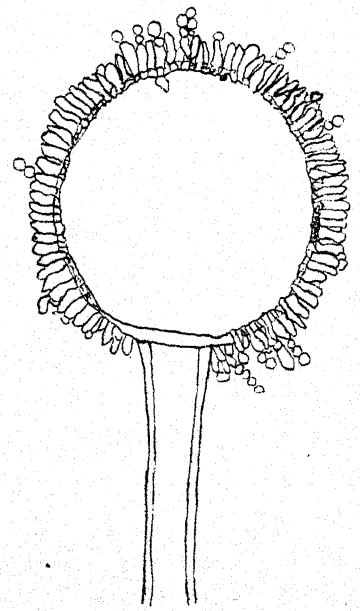

Fig. 1.—The head of *Aspergillus niger*. (Camera-lucida drawing. ×460.)

* Average of 120 readings.

The stalk.—The foot-cells were well differentiated with colourless walls submerged in the medium, (Fig. 2). Those of older stalks were provided with one or more stout projections which served as anchors (Fig. 3). The stalk was 500μ to 800μ long, 12μ broad,* nonseptate, with uniformly thickened smooth wall, light brown in colour slightly deeper below the vesicle. The brown colour originating a little distance above the foot-cell.

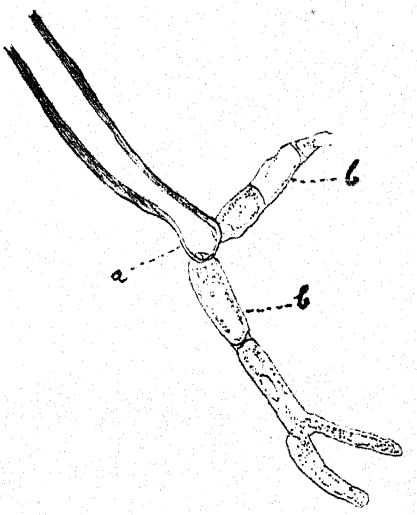

Fig. 2.—The base of the stalk of *Aspergillus niger*. *a*—The foot-cell; *b*—The vegetative cells. (Camera-lucida drawing ×900.)

Conidia.—The conidia were globose; 3 μ to 4 μ† in diameter, pinkish, spinulose with the spines deeper in colour due to the colour bars.

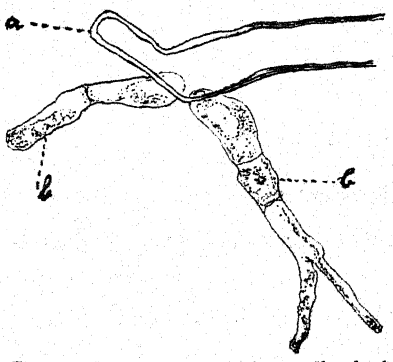

Fig. 3—The foot-cell of the stalk of *Aspergillus niger*. *a*—The anchor; *b*—The vegetative cells. (Camera-lucida drawing. ×900).

Sclerotium.—No sclerotia were noticed on rice medium or on Czapek and Richards' media.

Perithecia.—No perithecia were produced in culture over two months old.

IV. Inoculations.

In order to test whether the *Aspergillus* isolated from the decomposed apple was responsible for bringing about the rot, a series of inoculations were made of healthy apples of different varieties, with the pure cultures of the fungus. The results of one of the series carried out with the variety of apple known as the Amrhi from Kashmir are tabulated below (Table I). The apples for inoculation

* Average of 50 readings.
† Average of 250 readings.

were dipped in mercuric chloride (0·01 per cent. solution) for fifteen minutes and then washed repeatedly in sterile water. They were kept in deep covered dishes containing wet blotting paper, which was previously sterilized in the autoclave. Stab inoculations were made by placing loopfuls of the mycelium on the surface of the apple and stabbing it through the mycelial mass to depths of about half an inch by means of a sterilized needle. In the slit or incision inoculations the loopful of mycelium was inserted inside the incision, cut to a depth of about half an inch by a sterilized scalpel. Slabs were inoculated by smearing a loopful of the fungus growth on slices of apple about a quarter inch thick, cut under aseptic conditions, and placed in sterilized moist chambers. For each set, controls were started, in which similar operations were done, except inoculating with the fungus. In the last series the loopfuls of mycelial masses were placed on the uninjured surface of the apple.

Every one of these inoculations, in which the fungus was introduced through wounds in the skin, the rot was successfully induced, the controls remaining unaltered. In the last set, however, where the fungus was placed on uninjured surface of the apple there was no rotting. The fungus was recovered from these rotted spots and in every case it agreed with the morphological characters of *Aspergillus niger* with which they were inoculated.

TABLE I.

Results of inoculation of the apple with Aspergillus niger.

Variety of apple :—Amrhi (Kashmir).

Temperature :— { average mean from Nov. 15 to Nov. 25, 70° F.
,, ,, ,, ,, 26 to Dec. 5, 65° F.

Date of inoculation :—Nov. 15.

Nature of inoculation	Progress of rot					Recovery of fungus by reisolation
	Nov. 16	Nov. 21	Nov. 28	Dec. 5	Dec. 12	
I—Inoculation—						
Stab No 1	Slightly water-soaked round the stab.	Brown spot 5 mm. diam.	Same 1 cm., slight zonation at periphery.	Same 4 cm. diam., skin wrinkled.	All coalesced. More than half the apple has rotted. The skin is wrinkled.	Reisolated on Czapek medium on Dec. 12. Result,—Definitely positive. Spores appeared in the middle of the colony on Dec. 15.
,, ,, 2	Slightly water-soaked round the stab.	Brown spot 5 mm. diam.	Same 5mm. diam., no zonation.	Same 5 cm. diam., skin wrinkled.		
,, ,, 3	Slightly water-soaked round the stab.	Brown spot 2 mm. diam.	Same drying up.	Same 1 cm. diam., skin wrinkled.		

TABLE I—*contd.*

Nature of inoculation	Progress of rot					Recovery of fungus by reisolation
	Nov. 16	Nov. 21	Nov. 28	Dec. 5	Dec. 12	
Control—Stab	Dry and brown. No change in surrounding tissue.	No change .	No change .	No change .	No change.	
II—Inoculation—						
Incision No. 1.	Browning about 1 mm. both sides of the cut.	Brown spot elliptical about 1 cm. in middle.	Same 1 cm. in middle.	Coalesced. Combined diam.—5 cm.	All coalesced. Nearly half the apple has rotted.	Reisolated on Czapek medium on Dec. 12. Result,—Definitely positive. Spores appeared in the middle of the colony on Dec. 14.
„ „ 2.	Browning about 1 mm. both sides of the cut.	Brown spot elliptical about 2 mm. in middle.	Same about 5 mm. in middle.			
„ „ 3.	Browning is more than 2 mm. wide.	Brown spot elliptical about 3 mm. in middle.	Same 3 mm. in middle.	Same—1. 1·5 cm.		
Control—Incision.	Dry. No change beyond the cut.	No change .	No change .	No change .	No change.	
III—Inoculation—						
Slice No. 1 .	Soft below the infection drop.	Slice completely rotted, dark-brown colour.	Spores appearing in centre.	Covered with spores.	..	Reisolated on Czapek medium on Nov. 21. Result,—Definitely positive. Colony thickly sporulating.
„ „ 2 .	Soft below the infection drop.	Slice completely rotted, dark-brown colour.	Spores appearing in centre.	Thickly covered with spores.	..	
„ „ 3 .	Soft below the infection drop.	Slice completely rotted, dark-brown colour.	Dried up .	..	..	
Control—Slice	No change .	No change .	No change .	No change .	No change.	
IV—inoculation—						
Uninjured No. 1	No change .	No change .	No change .	No change .	No change.	
„ „ 2	„ „ .	„ „ .	„ „ .	„ „ .	„ „	
„ „ 3	„ „ .	„ „ .	„ „ .	„ „ .	„ „	

Thus it is conclusively proved that the kind of soft rot of stored apples in the United Provinces, described in this paper, is caused by *Aspergillus niger**. The fungus is unable to make its way through uninjured skin-surface and can cause the rot only when it reaches the flesh through broken surface of the skin, like bruises or insect punctures.

Plate L, fig. 2 is a photograph, taken on the twentieth day, of one of the apples inoculated with the fungus. The inoculation was done by incision at three places marked with crosses in the figure. The three rotted areas coalesced into one big spot. There was no fruiting of the fungus on it.

V. Action of the fungus on host tissue.

It has been shown that *Aspergillus niger* is incapable of penetrating through uninjured skin of the apple. But once it reaches the interior of the fruit it grows rapidly after the first few days, bringing about quick dissolution of the cell wall and break-down of the tissue. This action takes place in advance of the invading hyphæ, for although near the periphery of the rotted spot the tissue becomes quite soft and pulpy, there is little of the mycelial network. The softening is brought about probably by an enzyme secreted by the invading hyphæ. That the mycelium of *Aspergillus niger* contains an active principle capable of dissolving the host tissue was proved by treating pieces of apple with the mycelial extract. The latter was prepared by William's [1915] method. Three days old cultures of *Aspergillus niger*, in Richards' solution, was dried in the sun and thoroughly pounded with clean washed sand. The mycelium in mass was slimy and gelatinous in consistency. When pounding with sand it gave off a pungent smell. The pounded mass was soaked in water for an hour with frequent stirring. It was then centrifuged and the supernatant liquid poured off. In this liquid were dipped discs of the apple core about a centimeter in diameter, cut out with a clean cork borer, and sliced off to the thickness of a millimeter each. Some discs were also kept in Richards' solution as the control. Within four hours, those in the fungus extract became soft, and after twenty hours changed into a pulpy mass. Under the microscope the loose cells could be seen floating in the drop. The control discs were quite intact and remained firm and spongy even after twenty-four hours. Thus an extract of the mycelium was obtained which was able to act on the cell wall dissolving the middle lamella and letting loose the cells of the tissue. The extract was destroyed by heating to 70°C. There was indication, therefore, that the active agent in the

* Since sending the paper for publication, an abstract of a paper entitled "Diseases of Kashmir Apples" by Pushkar Nath (Abstracts of papers Indian Science Congress Sec. 5 Bot. 1933) has been published. He isolated an *Aspergillussp.* from an apple rot and succeeded in establishing its pathogenicity.

extract was of the nature of enzyme. The softening of the host tissue in the water-soaked area around the rotted spot, where the mycelium was not abundant, might be due to the action in advance of the same enzyme secreted by the growing hyphæ. In the region thus killed and softened by its enzyme, the mycelium penetrated more easily. That the progress of the fungus is dependent on killing in advance of the surrounding tissue is further proved by the fact that in all inoculation experiments the progress of the rot during the first few days (Table I), when the mycelial growth was not great, was rather slow, while it spread more rapidly later on, when the mycelium had grown bigger and was secreting the enzyme in sufficient quantity to cause softening of the surrounding host tissue over larger area.

VI. Reaction of the medium on the growth of the fungus.

It has been mentioned before that the sour varieties of apples are found to be more susceptible to the soft rot. To examine whether this susceptibility is conditioned by the acidity of the substratum, a series of plate cultures of the fungus was made in Czapek solution of different acidity. This solution, as prepared, was neutral. To it were added different quantities of normal malic acid to bring the reaction varying from +2·5 to +25 (Fuller's scale). The results are given in Table II.

Table II.

Rate of growth of Aspergillus niger *in media of different acidity.*

(Number of cultures—6. Date of inoculation—24th November 1932. Temperature—max. 76°F., min. 62°F.)

Reaction of medium (Fuller's scale)	Average diameter of colonies in cm. on			
	28-11-32	29-11-32	30-11-32	1-12-32
Neutral	2·24	2·78	4·35	5·2
+2·5	2·46	3·4	4·84	6·2
+5	2·74	3·48	5·1	6·6
+10	3·12	4·74	6·56	8·25
+15	3·36	5·72	7·76	9·0
+20	3·52	6·12	8·1	Completely filled 9 cm. Petri dishes.
+25	3·48	6·68	8·65	

This table gives the rate of growth of the fungus on neutral to +25 media. In this series 2 per cent. agar-agar was added in each, but due to the strong acid nature of the solution, Nos. 5, 6 and 7 did not solidify. In this table the average measurements of the colonies in six different plate cultures are given. It is clear from this table that the fungus prefers strong acid medium.

That this marked increase in growth in the stronger acid ranges might not be due to the liquid nature of the medium, and also to make an effort to find out the amount of acidity providing the optimum condition for growth, another series of plate cultures were made (Table III). In this series no agar-agar was added. The range of acidity varied from +5 to +40. Six plates were prepared for each of the series and the measurements given in the table are again the average diameter of six colonies from six different plates.

In both these experiments the cultures were, in every case, made in Petri dishes with internal diameter of 9 cm.; each containing 10 c.c. of the culture medium.

TABLE III.

Rate of growth of Aspergillus niger *in media of different acidity.*

(Number of cultures—6. Date of inoculation—2nd December 1932. Temperature—max. 72°F., min. 60°F.)

Reaction of medium (Fuller's scale)	Average diameter of colonies in cm. on			
	6-12-32	7-12-32	8-12-32	9-12-32
+5	0·62	0·76	0·92	1·28
+10	0·83	1·53	1·77	2·0
+15	0·9	1·73	2·0	2·2
+20	0·95	1·75	2·0	2·2
+25	1·37	2·04	2·36	3·8
+30	1·53	2·07	2·3	3·6
+35	1·5	1·78	2·04	2·56
+40	1·1	1·44	1·8	2·0

In this series also the maximum growth was obtained in +25 and +30 solutions; and since all the solutions in the series were of the same density, greater growth at +25 in the last series was not due to the liquid nature of the medium, but to its being more strongly acid than the others. From Table III it is also clear

that with the increase of acidity beyond +30 the rate of growth decreases. Thus the optimum reaction of the medium for this *Aspergillus niger* lies between + 25 and +30. The pH value of these solutions has been determined to be 3·8 and 3·4 respectively. Webb [1919] found that the spores of *Aspergillus niger* showed a maximum of germination in *M*/5 mannite solution at pH 2·8-3·1. The present experiments have proved that the medium with hydrogen-ion concentration lying between 3·4 and 3·8 is also the most favourable for the mycelial growth of *Aspergillus niger*. Hence the greater susceptibility to rotting of some varieties of apples by *Aspergillus niger* depends on their sourness.

Less rapid growth in the second series of these plate cultures, as compared to the first, may be explained by the fact, that in the second series the major portion of the mycelial growth, which appeared like a thick gelatinous mass, sank and was lying immered in the watery solution, and was not therefore growing in the optimum condition of oxygen supply. In the first series on the other hand, although Nos. 5, 6 and 7 remained liquid, their consistency was thicker and the colonies were mostly floating on the surface. Also the mean temperature of the laboratory during the second series was lower by about 4°F.

VII. Oxygen relation.

That plentiful supply of oxygen is necessary for the growth of *Aspergillus niger* was demonstrated by making cultures on Czapek medium according to Wright's [1900] method of anaerobic cultures for bacteria. Three slants of the medium in test tubes were thickly sown with the spores from a two-week old culture. The cotton plugs were pushed in about an inch and then soaked with 2 c.c. of freshly prepared 10 per cent. solution of pyrogallic acid, over which were next poured 2 c.c. of saturated solution of caustic soda. The mouths of the test tubes were immediately closed with tight-fitting rubber stoppers and sealed with melted paraffin. The oxygen present in the tubes being absorbed by the mixture of pyrogallic acid and caustic soda, none of it was available to the spores. Even after a week these tubes did not show any growth, while untreated (control) tubes had large freely-sporing colonies within four days.

VIII. Control of the rot.

The above study of the behaviour of *Aspergillus niger* in causing soft rot in apples has shown that the fungus is unable to penetrate into the apple except through the injured skin. Its prevention, therefore, lies in careful handling of the fruit to avoid bruises. It is suggested that particular care should be taken at the time of picking. The fruits should not be allowed to drop on the ground, but be gently lifted by hand and carefully placed in baskets or boxes, the sides and bottom

of which are padded with dry moss. Before storage they should be examined once again and each wrapped separately in tissue paper. For export the wrapped fruits should be tightly packed in dry moss in boxes to prevent shaking and rubbing.

IX. Acknowledgments.

In conclusion we wish to express our thanks to Mr. G. R. Saxena for finding out the pH value of the media, and to Mr. P. B. Richards for reading the manuscript.

X. Summary.

A very large proportion of the apples in the markets in the United Provinces are damaged by a kind of soft rot. The sour apples are more susceptible.

Aspergillus niger was isolated from these rotted apples.

Inoculation through wounds of sound apples with pure culture of this *Aspergillus niger* produced the characteristic rot within a short time. The fungus was successfully re-isolated from it.

The fungus was unable to enter through uninjured skin of the apple.

The softening of the flesh of apple is due to an enzyme secreted by the invading hypha. The progress of the latter depends on killing in advance of the surrounding tissue by this enzyme.

The greater susceptibility to rotting of some varieties of apples by *Aspergillus niger* depends on their sourness. The optimum reaction lies between + 25 and + 30 of malic acid.

Aspergillus niger is strongly aerobic.

Prevention of the soft rot lies in careful handling of the fruit to avoid bruises.

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PRELIMINARY INVESTIGATIONS ON THE DISEASES OF BANANAS OCCURRING IN THE PUNJAB AND THEIR METHOD OF CONTROL.

BY

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(With Plates LI-LIII and three text-figures).

INTRODUCTION.

Owing to the peculiar climatic conditions of the province, the cultivation of bananas in the Punjab has not been carried on to any great extent. Nevertheless the increasing importation of this fruit from other parts of India clearly shows that there is a large demand for it in this province. The efforts of the Department of Agriculture have been in recent years concentrated on finding out some suitable varieties by trials of bananas collected from all over India and even outside.

During the summer of 1932, some diseases made their appearance in the experimental banana plot, in the Botanical Area at Lyallpur. Survey of the fruit markets and fruit stalls of some of the important towns (Lyallpur, Lahore, Jhelum) revealed that a high percentage of bananas, mostly imported, were also affected with such diseases, showing that these are most probably present in other banana-growing parts of India as well. Subsequently it was observed that these diseases occurred in the banana plantation in Amritsar and Lahore.

This paper embodies some preliminary observations made on the various diseases, their causal organisms and their methods of control, both in the plantation and storage.

MATERIAL AND METHODS.

The banana plantation from which material for this investigation was taken was planted in 1930, and occupies about one acre. It consists of over 80 varieties of bananas including both culinary and ' table ' varieties.

A survey of the plantation in July 1932, revealed the existence of the following different types of diseases :—

(1) Main stalk rot.
(2) Black-tip or finger-tip.
(3) Pseudo-stem rot.
(4) Leaf-spot.
(5) Peculiar curvature of the midrib and crumpling of leaves.

In addition to these the following diseases were observed in the " curing pit " and storage.

(*a*) Green ripeness.
(*b*) Stem-end rot.
(*c*) Finger-stalk rot.
(*d*) Unsightly skin blemishes.

By the usual mycological methods, a very large number of isolations were made. Those fungi which appeared most frequently were taken up, purified and tried on bananas under controlled conditions to determine the active causal agent. In almost every case a species of *Gleosporium* or that of *Botryodiplodia* was obtained associated, at times, with some Fusaria. No successful inoculations were obtained with any of the *Fusarium sp.* thus isolated. It is possible that these fungi may be accentuating the attack when once it is started. To ensure purity of cultures for further work, the start was made in each case by Brown's single hyphal tip method [1924] and the stock cultures were kept on the slants of potato extract agar.

For inoculation purposes, the bunches were cut up into " hands," washed, sterilized in 1/1,000 mercuric chloride solution for 10 minutes, followed by thorough washing with several changes of distilled sterile water, allowed to dry and then inoculated with the required fungi by Granger and Horne's plug method [1924]. The inoculated bunches were wrapped up in sterilized tissue papers after smearing the cut ends of the main stalk with vaseline. The bunches were placed at the desired temperature in incubators.

For cultural study the following media were used :—

(1) Richards' agar.
(2) Potato extract agar.
(3) Brown's synthetic agar.

To avoid confusion it may be mentioned that the terminology followed is as given below :—

1. " Finger "	. .	Individual banana fruit.
2. " Finger stalk "	.	The short stalk of each banana fruit.
3. " Hand "	. .	A cluster of individual " fingers ".
4. " Cushion "	. .	Where several finger stalks meet to form a " hand " and these cushions project out from the main stalk of the whole bunch.
5. " Main stalk "	. .	The central axis.

Observations.

The diseases generally met with may be divided into :—

(i) Diseases in the plantation.
(ii) Diseases in storage.

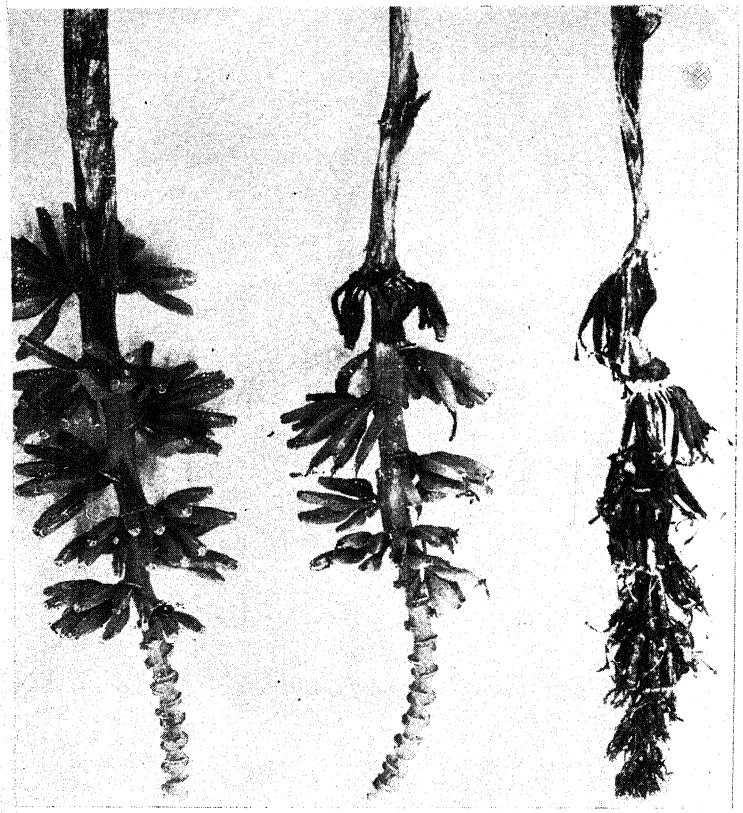


Fig. 1.—Showing three stages of main stalk rot.

Fig. 2.—A typical healthy secondary sucker.

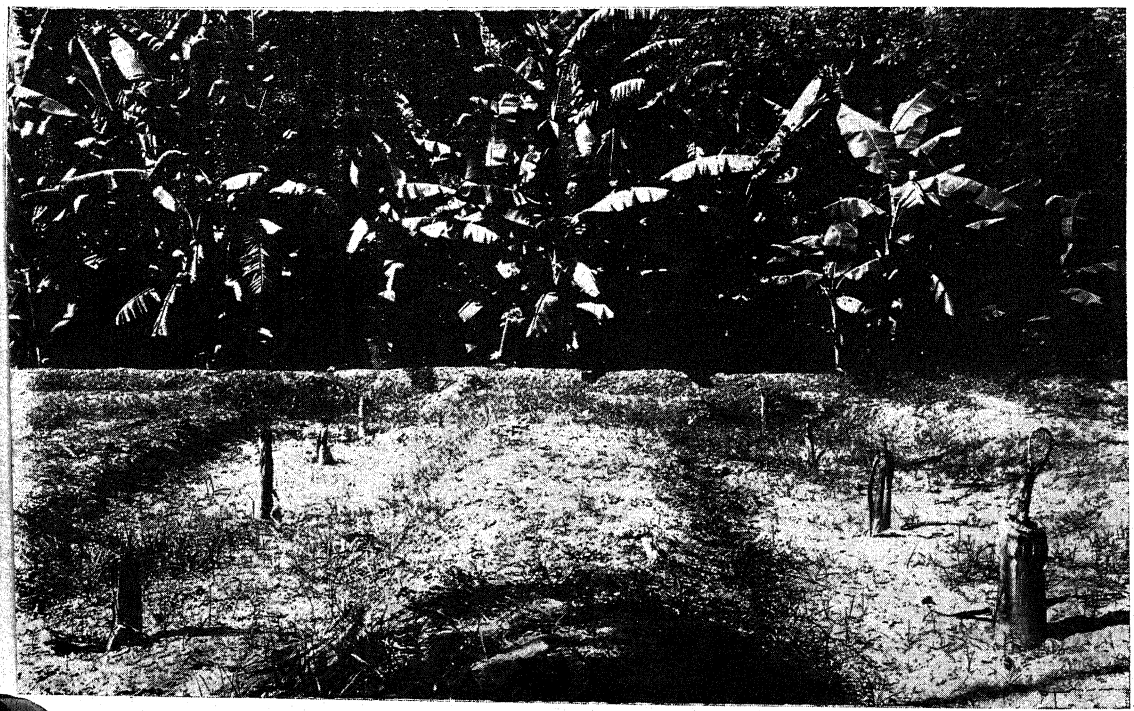


Fig. 3.—Two rows of suckers (2 months after planting) affected by pseudo-stem rot.

Plantation diseases.

Main stalk rot.—The disease originates as a dark brown patch from the central point of the marked curvature of the main stalk, formed as a result of the tremendous weight of the bunch hanging below. It then gradually spreads downward and passes on to the fruit and upwards as far as the point at which it emerges from the plant. The disease considerably retards the development of the fruit and weakens the main stalk to such an extent that the entire bunch, and, in some cases the main plant itself, gets blown down by strong winds, that are so frequent in the Punjab during the summer season. The affected tissue shrivels, dries up and shows considerable amount of shredding. In advanced stages the pustules of *Gleosporium* are often observed giving the surface a dull rusty appearance. It is more or less a dry rot, but when it extends to the pseudo-stem where it meets comparatively soft tissue with high moisture contents, it turns into wet rot and progresses very rapidly. At this stage the entire plant is liable to get blown down by even a mild wind.

If the infection starts during the early stages the entire bunch is destroyed. On the other hand if infection takes place at a comparatively later stage only the uppermost hand or two may be affected (Plate LI, fig. 1).

This disease is not so prevalent in the varieties having horizontal bunches, but unfortunately all our "table" varieties are of pendent bunch habit. None of the varieties present here showed immunity. In this case the causal organisms are *Gleosporium sp.* and *Botryodiplodia.* The disease has been reproduced with all its characteristic symptoms by artificial inoculation both in the laboratory and in the plantation.

"*Black-tip*" *or* "*finger-tip*".—The characteristic symptoms of the disease are the unsightly black appearance of the tip (distal end) of immature fruit, followed by a premature yellowing of the lower healthy portion. In advanced cases the pulp becomes soft and watery with a peculiar odour, while the skin becomes dark and wrinkled (Plate LII, fig. 1). At this stage numerous small black pycnidia can be observed emerging through skin, from which pure cultures of *Botryodiplodia* have been isolated. The extent of damage in some cases has been noticed to be as high as 20 per cent.

The source of infection is the persistent perianth. The disease originates in the decayed perianth or style from where it gradually spreads along the fruit causing a blackish discolouration of the skin. The causal organism has been often isolated from the persistent perianth.

In one experiment an apparently healthy immature bunch of bananas (culinary variety, with persistent perianth) was obtained from the plantation, and was cut into hands, which were then incubated in sterile moist chamber dishes. By the sixth day most of them had developed the typical characteristic black-tip. In ad-

vanced cases the tiny black pycnidia were in great abundance and even the dark grey hyphæ of the fungus were noticeable by the 10th day.

In order to obtain conclusive results another bunch of the same variety was obtained, cut into hands, sterilized in 0·1 per cent. mercuric chloride solution and were then inoculated with *Botryodiplodia* pure culture, by the 'plug' method. Two plugs were bored out on each finger. In one of the cavities of each an inoculum of the fungus was given, while the other was left as control. Besides these, two or three fingers in each hand were also left uninoculated. The inoculated bunches were placed in potato dishes containing moist thick blotting paper in the lid and bottom to maintain good humid conditions.

On the very next day a tuft of fungal hyphæ could be seen in the inoculated cavities. By the third day the attack had started and in another two days had developed the typical finger-tip disease, while the controls remained sound.

The original fungus was re-obtained from all these cases. This experiment was conducted at room temperature (28-30° C.).

In another experiment the fruits were inoculated in exactly the same manner, but were divided into two lots. In one of the lots there were no moist blotting papers in the potato dishes. One potato dish from each lot was placed at a temperature of 14-15°, 20°, 25°, 30°, 35° and 40° C. Maximum infection, as is evident from the table below, was obtained at a relatively high temperature under moist condition. This explains the prevalence of the disease in the plantation in the hot and humid months of rainy season (June, July and August).

TABLE I.

Effect of temperature and humidity on black-tip infection. (Duration experiment 10 days.)

	Dry	Moist	Percentage infection
14-15° C.	Dry	..	5
Do.	..	Moist	5
20° C.	Dry	..	10
Do.	..	Moist	12
25° C.	Dry	..	25
Do.	..	Moist	45
30° C.	Dry	..	10
Do.	..	Moist	75
35° C	Dry	..	10
Do.	..	Moist	90
40° C.	Dry	..	0
Do.	..	Moist	10

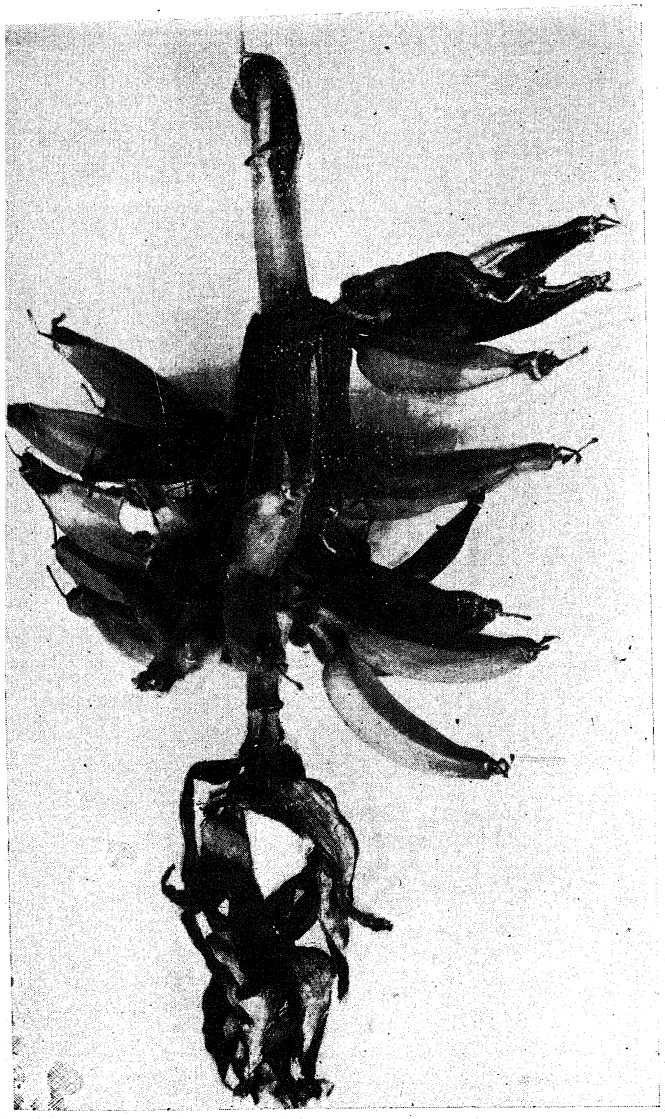


Fig. 1.—Bunch of bananas affected with finger-tip.

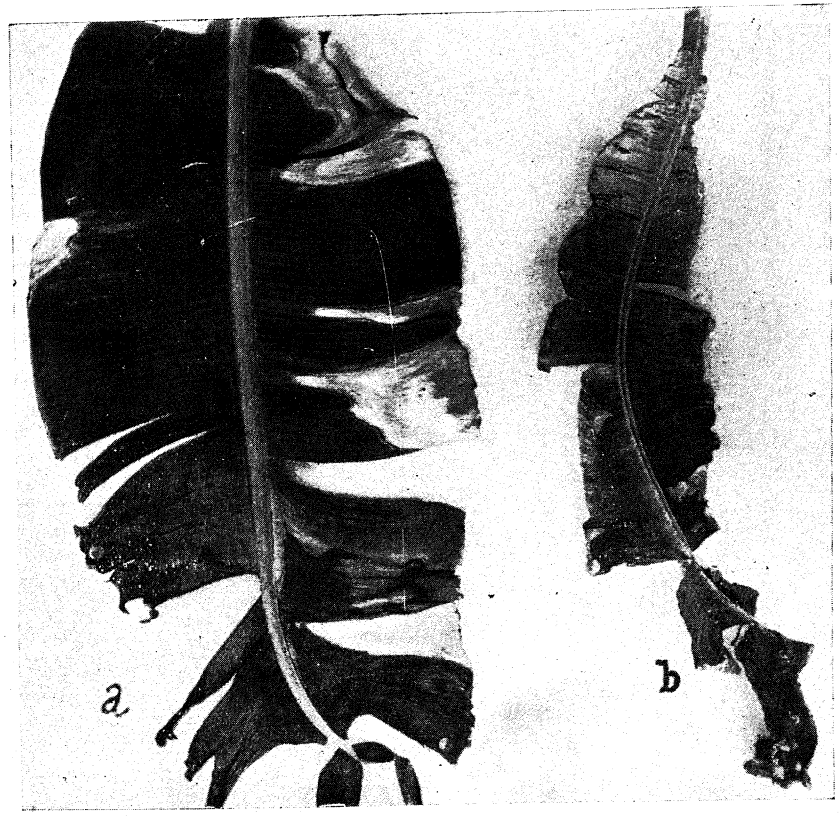


Fig. 2.—(*a*) Showing leaf-spot. (*b*) Showing peculiar curvature of mid-rip.

The difference in the extent of infection for "dry" and "moist" conditions is more marked at higher temperature. Whether this difference is brought about solely by a rise in the temperature or by the relatively high humidity is a question that needs further investigation.

The sudden decrease in the number of fingers affected at 40° C. under "dry" conditions may be merely due to the drying up of the inoculum.

Pseudo-stem rot.—This is one of the most serious troubles of the plantation (Plate LI, fig. 3). The disease causes the rotting of pseudo-stem of the young suckers planted for propagation, destroying completely the central growing bud. Among the suckers planted in July, August and September of 12 different varieties as many as 60-80 per cent. failed to come up. The data are represented in the table below:—

TABLE II.

Date of planting	Variety	No. of suckers planted	No. failed to grow (pseudo-stem rot)	No. growing	Remarks
28th August 1932	"Guilthlaheim"	5	5	0	
,, ,, ,,	"China chumpa"	19	17	2	Just struggling along.
,, ,, ,,	"Amritsar"	5	2	3	Only 1 healthy, 2 merely struggling on.
,, ,, ,,	"Amartaman"	15	5	10	Only 3 healthy.
,, ,, ,,	"Rawalpindi"	5	0	5	Only 1 healthy.
,, ,, ,,	"Dorobalai"	15	14	1	1 fairly healthy.
,, ,, ,,	"Saharanpur 81"	3	3	0	
,, ,, ,,	"Saharanpur 79.'	3	0	3	Just struggling along.
,, ,, ,,	"Ramkela"	5	5	0	
,, ,, ,,	"Ilachi"	5	2	3	Just struggling along.
,, ,, ,,	"Elsi"	4	2	2	Only 1 fairly healthy.
,, ,, ,,	"Kanai bansi"	10	5	5	Only struggling along.
,, ,, ,,	"Rajpuri"	5	4	1	1 healthy.

The extent of damage is markedly greater in suckers, which were topped before planting.

Gleosporium and *Botryodiplodia*, along with some bacteria have been frequently isolated from the affected suckers.

It has been observed in practically every banana afflicted with this malady, that the disease is confined only to the pseudo-stem and does not affect the corm, the real underground stem. This being so, the control of the disease lies in the complete severance of the pseudo-stem from the corm, and planting the latter after dipping in some suitable disinfectant (2 per cent. copper sulphate has proved very effective).

In one typical experiment 24 young suckers were obtained from the plantation. Half of them were topped in the usual way leaving about 18 in. of trunk above the corm and planted immediately in pots. The other half were severed off in level with the corm, treated with 2 per cent. copper sulphate solution for 10 minutes, dried in the sun and then planted into pots, filled with the same soil as the other half. All of them were placed in the glass house and watered every morning.

The treated ones gave perfectly healthy secondary suckers and in some cases even healthy primary suckers. Only two out of these 12 failed to come up while in the untreated lot 10 died of the pseudo-stem rot and the remaining 2 seemed merely struggling along.

In view of above results, it appears that the most feasible way of establishing healthy plantation is either by selecting young healthy secondary suckers (Plate LI, fig. 2) or by selecting disease-free corms, which should be treated as described above, prior to planting.

Leaf-spot.—This disease was noticed to be particularly severe in *M. Cavendish* variety and to a less degree in several others. The symptoms of the disease are localised, light brown dry patches with a bright yellow margin which gradually spread and destroy the entire leaf tissue affected (Plate LII, fig. 2 *a*). The most peculiar characteristic of this disease is the occurrence of the disease spots invariably on only one-half of the leaf blade, near the margin. This is most probably due to the fact that infection takes place before the leaf unfolds itself. As the leaf is rolled up at this stage, it is but natural that only the half lamina on the outside should get infected.

This disease having been found to affect, so far, mostly those varieties which have not given any indication of thriving under the Punjab conditions, no attempts have been made to isolate the causal agent and study it in more detail.

Peculiar curvature of the midrib.—The symptoms of the disease consist in a peculiar curvature of the midrib of a few leaves. The leaf blades also exhibit peculiar distortion in parts, where the veins lose their natural parallel position. The affected portion is definitely of a lighter green colour indicating partial loss of chlorophyll contents and is comparatively more brittle (Plate LII, fig. 2*b*).

In this case as well, no further study has been made since affected varieties have not proved promising under local conditions.

Storage diseases.

Green ripeness is that stage of the fruit when it fails to develop the proper ripe yellow colour. The fruit remains green or at the most a shade of dull yellow, while the pulp within gets soft to the ripe or even over-ripe stage without developing the requisite flavour or taste. Repeated attempts at isolation from affected fruits have failed to yield any fungal or bacterial organisms. As the disease has been invariably found to occur during exceptionally hot summer days both in the plantation and the curing pit, it is reasonable to assume that it is merely a physiological break-down attributable to high temperature. This view has been confirmed in various curing trials carried out at an abnormally high temperature of 45°-50°C. ; while hands from the same bunches cured at lower temperature ranging from 15°-40°C. were free from this disease.

Stem-end rot.—This is a very serious disease met with in the curing pit. The disease starts from the cut end of the peduncle, spreads rapidly down the entire length of the stalk rendering it black and soft, passing at the same time into the cushion, finger stalks and fingers causing complete rot of all in severe cases. The disease has been found to exist to varying extent invariably in all the bunches in the curing pit at Lyallpur as well as the various banana store-houses visited at Lahore, Lyallpur and Jhelum. As both local and imported varieties have been found to be affected, there is reason to believe that this disease must be prevalent throughout the country.

The fungi responsible for this disease are *Gleosporium* and *Botryodiplodia*. Repeated isolation from the diseased material almost always yielded these two organisms. At an advanced stage of attack it is not uncommon to see the salmon or rusty coloured acervuli of Gleosporium on the dead parts or the numerous tiny black pycnidia of Botryodiplodia breaking through the skin.

Under controlled laboratory conditions with pure cultures of *Gleosporium sp.* and *Botryodiplodia sp.*, the disease has been successfully reproduced with all its characteristic symptoms.

A typical experiment is described below.

Two bunches of the same variety and practically of the same age were cut into individual hands, with 3-4 inches of peduncle with each, sterilized and inoculated with *Gleosporium* or *Botryodiplodia* on the central axis on one end. Two of these hands (one from each bunch) were left uninoculated to serve as controls. All these bunches were placed in moist chamber dishes and incubated at 28°-30°C.

The disease with all its characteristics developed in all the cases, attacking the main stem to start with, rendering it soft and black, and gradually passed into the

cushions and thence to finger stalks (Plate LIII, fig. 1). The controls remained sound all the time.

This disease is not confined to any particular variety. It affects all the varieties growing in the experimental plantation more or less to the same extent.

The experiments under controlled conditions as well as the observations made have clearly brought out the fact that temperature is one of the main contributive factors to the damage caused by these fungi.

Two lots consisting of six hands each were inoculated with the two fungi and wrapped in tissue paper. One hand from each lot was placed at 15°, 20°, 25°, 30°, 35°, and 40°C. An uninoculated hand was also kept at each of these temperatures for control purposes.

The progress of disease at the end of 10 days is graphically represented below. The controls remained sound in all the cases.

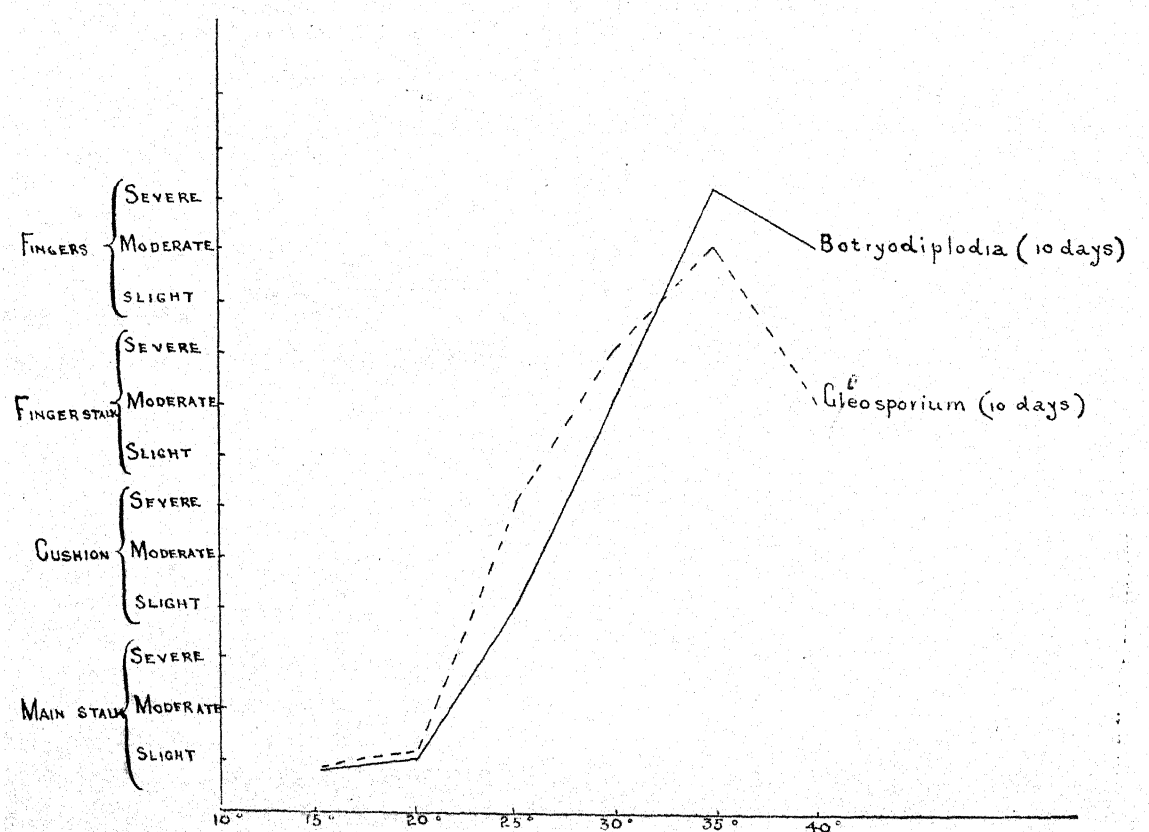
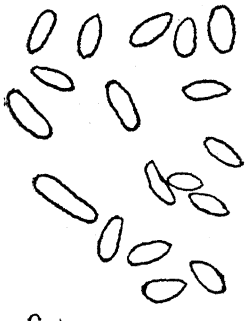
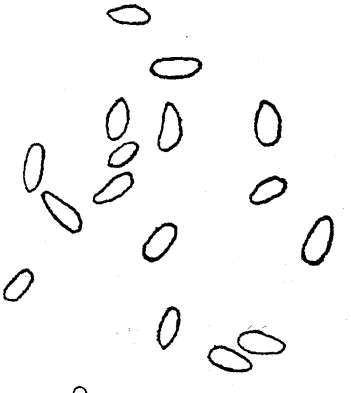


Fig. 1. Showing effect of temperature on storage rot of bananas (Gleosporium and Botryodiplodia).

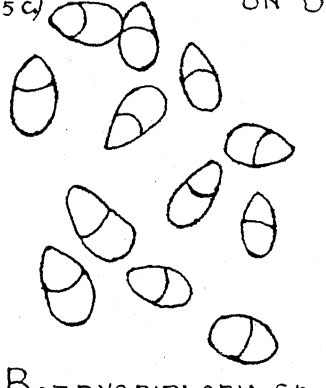
Fig. 1.—A "hand" showing typical attack of Gleosporium progressed to the finger-stalks.



GLEOSPORIUM SP. ×440
(RICHARD'S AGAR AT 25°C)



GLEOSPORIUM SP. ×440
ON BANANA FRUIT 25°C.



BOTRYODIPLODIA SP. ×440
(RICHARDS' AGAR 25°C)

It is clear from Fig. 1 that the spread of the disease is more or less in direct proportion to the temperature. From a slight attack of the disease observed in the bunch at the low temperatures of 15° and 20°C. it has progressed to the cushion at 25°, to the fingers at 35° and 40°C.

The above results go to confirm the observations made in curing pit where the severity of the attack is very pronounced during the hot summer months of May to August.

The practical application of the above results in the curing of fruits, as well as in finding out the proper season of planting suckers to obtain mature fruit at the most favourable period of the year, are questions of undoubted importance and require to be emphasised.

In the matter of preliminary treatment of the bunches prior to carrying them to the curing pit, various trials conducted have proved the efficacy of smearing the cut end of the bunches with a thin coating of vaseline, preferably wax and vaseline mixture, and wrapping them with sterile grease-proof paper in the manner suggested by Tomkins [1931]. It is therefore recommended that the bunches should be subjected to this treatment before sending them to the curing pit.

Unsightly skin blemishes.—These blemishes occur on fingers when exposed to air after they are removed from the curing pit. Even the most attractive yellow fruit develops these blemishes within an hour or two of removal from pit, rendering them most unsightly.

Observations made in the curing pit as well as the results obtained under controlled conditions have revealed the fact that these blemishes are mainly caused when the temperature at the time of curing is comparatively high. The fruits cured at 35°-40°C. have invariably developed the symptoms after a brief exposure to the air while the hands from the same bunch cured at lower temperatures of 15° and 20°C. remained free from blemishes for about two days.

Finger stalk rot.—It is merely an incidence of localized infection, caused chiefly by *Gleosporium sp.* It does not cause as much damage as stem-end rot. The disease causes rotting of the finger stalks which results ultimately in shredding of fruit thus reducing its market value.

CULTURAL CHARACTERS OF THE CAUSAL ORGANISMS.

The two fungi *Gleosporium* and *Botryodiplodia* were grown on Richards' agar, potato extract agar and Brown's agar at 10°, 20°, 25°, 30°, 35°, and 40°C. All the three media were found suitable for their growth, Richards' being the best, on which the growth was luxuriant and sporulation profuse and comparatively early. Rate

of growth was determined by measuring the diameter of the colonies from day to day. The results (average of 4 plates) are represented graphically below (Figs. 2 and 3).

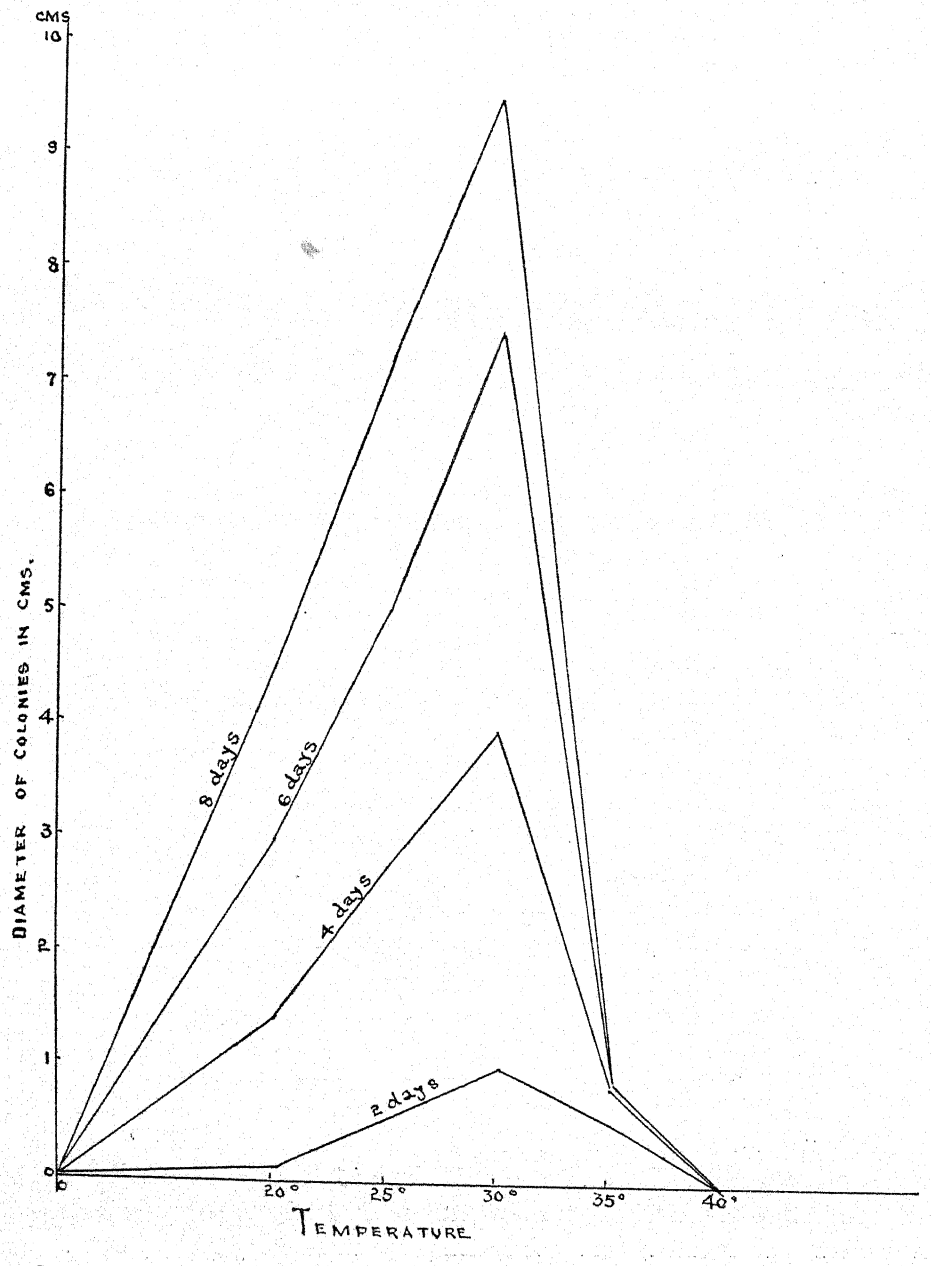


Fig. 2. Showing effect of temperature on rate of growth (*Gleosporium sp.*) on Brown's agar.

Gleosporium sp. has very sparse mycelium and the colonies are thin and creeping. Gradually with the formation of spores they become bright salmon pink in colour and sporulation is profuse. The spores are non-septate, sub-hyaline, ovoid, with both ends almost alike and are usually vacuolate.

Botryodiplodia is a very fast growing fungus and has profuse aerial mycelium which is cottony white to start with, but gradually turns greenish grey and finally dark grey when the cultures are about three weeks old. Countless tiny black pycnidia partly embedded in the medium are formed. On the comparatively weaker media (potato extract and Brown's agar) pycnidial formation is both late and less abundant. Black sclerotial bodies are formed frequently in old cultures.

The spores are ovoid, non-septate and sub-hyaline when young, but with age they become dark brown and develop a septum in the middle. One end is slightly narrower than the other and on this end they are attached to the inner wall of the pycnidium with short strands. Interspersed in these conidia bearing strands, are sterile, long, slender hyphæ.

Typical spores of both Gleosporium and Botryodiplodia are shown in Plate LIII, fig. 2. Their dimensions and mode (per count of 100 spores) are given below (Richards' agar at 25°C.) :—

Fungus	Range	Average	Mode								
			Length					Breadth			
			0 to 10	10 to 15	15 to 20	20 to 25	25 to 30μ	0 to 5	5 to 10	10 to 15	15 to 20μ
Gleosporium sp.	11 × 5μ to 24 × 8μ	15 × 6μ	0	52	42	6	0	10	90	0	0
Botryodiplodia sp.	16 × 12μ to 27 × 13μ	20 × 12 μ	0	0	42	56	2	0	0	100	0

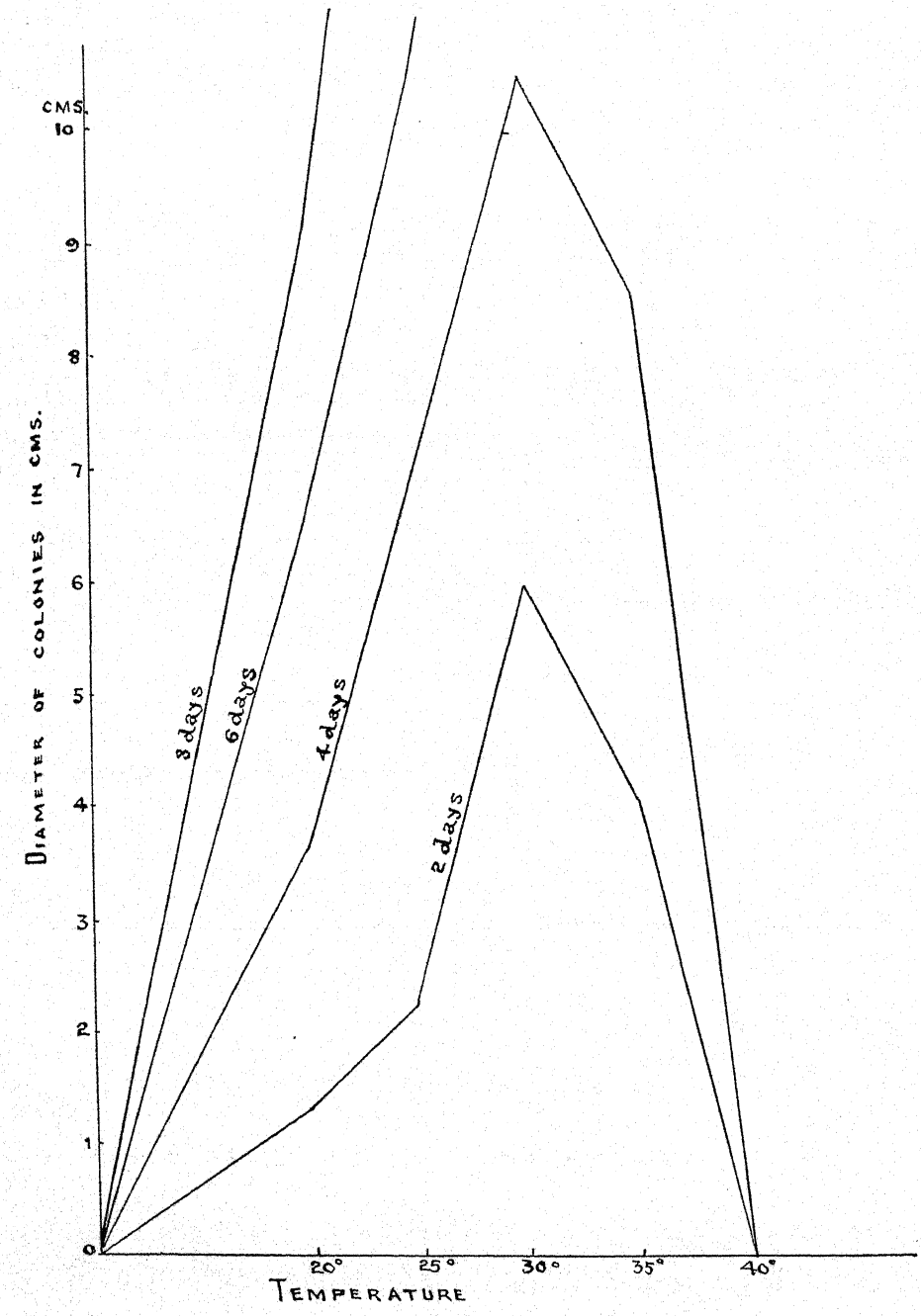


Fig. 3. Showing effect of temperature on rate of growth (*Botryodiplodia sp.*) on Brown's agar.

For the other two media tested (Richards' and potato extract agar), similar growth curves were obtained.

Though the optimum temperature for growth of both the parasitic fungi is 30°C., yet the attack on the host is more vigorous at 35°C. This can probably be attributed to the lowered resistance of host tissue at higher temperature. Vasudeva [1930], working with apples has similarly found a marked fall in the resistance of the host at high temperatures.

Discussion.

The data given on the foregoing pages sets forth the preliminary observations made on the various important banana diseases occurring in the plantation as well as in the curing pit and storage. Similar diseases have been described by other investigators in different parts of the world. Agati [1922] from Philippine Islands, Ashby [1912] from Jamaica, Ogilvie [1928] Bermuda, Campbell [1925] Fiji Islands, Carpenter [1918] Hawaii, and Laubert [1926] from Germany. The recent and comprehensive work of Tomkins [1931] at Cambridge and of Wardlaw and McGuire [1931, 1 & 2] at Trinidad, deals however mainly with transport and storage problems. They have adduced valuable evidences with regard to the control of banana diseases, occurring mainly in Gros Michael and Cavendish varieties. A knowledge of varietal susceptibility to different diseases, the extent of kind and damage caused, the methods of infection and the varying responses of the causal organisms to the different environmental factors, as well as the methods of control are a few of those important problems that are by no means uniform in various banana growing tracts of the world. With the exception of Dastur's [1916] work on ripe rot, Basu's [1911] on a kind of wilt in Bengal, and Mitra's [1930] report on a species of Helminthosporium attacking bananas in C. P., there does not exist any literature on the diseases of Indian bananas. The present contribution is expected to throw some light on this important subject. But owing to the wide range of diseases under study, the results obtained can hardly claim to be comprehensive.

A complete study of any disease organism is intimately connected with a thorough knowledge of the host plant. In this connection, however, the lack of any reliable botanical classification of Indian banana varieties has proved to be a great drawback. As has been emphasised by Cheesman [1931] a closer understanding of the various problems connected with banana culture as well as its diseases can only be achieved when this work of classification is completed. The attention of our horticulturists and botanists is earnestly solicited on this important aspect of the question.

The object of the banana grower resolves itself into two important separate heads :—

1. The propagation of healthy suckers.

2. The successful curing of fruit free from disease.

It has been shown in this study that the former can be achieved by separating from affected suckers, the under-ground stem or corm, which is usually free from infection, and planting the same after dipping in 2 per cent. copper sulphate solution. The secondary suckers coming out of such corms, being disease-free, would form the nucleus of the healthy plantation.

As to the diseases in curing pit, it has been shown that the chief factors responsible for the damage are high temperature and high humidity. Various trials conducted have revealed that the most favourable temperature for successful curing is 15°-20°C. (59°-68°F.). Wardlaw and McGuire [*l.c.*] in their work on storage and transport of bananas have also found that at lower temperatures the diseases remain under a strict check. The low temperature curing coupled with vaseline treatment would therefore point out a successful method of checking the various storage diseases and of curing fruit with its natural flavour and aroma developed to its best.

In the plains of the Punjab, the prevailing temperature in the curing pit during the months of June, July and August being about 35°-40°C., it is obvious that these months can hardly be termed to be suitable for affecting a successful check on the growth of various disease organisms. The method of varying the planting season of suckers in order to bring them to fruit at a season when the most favourable temperature prevails in the curing pit is a point that will be elucidated in a later paper.

The author wishes to express his thanks to the Department of Agriculture, Punjab, for affording the necessary facilities for carrying out this investigation and specially to Professor J. C. Luthra for his keen interest and helpful suggestions. The writer is also indebted to the Fruit Specialist for providing the material and to Mr. K. C. Naik for valuable criticism on the horticultural aspect of the problem.

Summary.

1. Various diseases of bananas occurring in the plantation and curing pit have been briefly described.

2. Most important of these have been studied at some length along with their causal organisms, which are species of Gleosporium and Botryodiplodia.

3. Effect of temperature and humidity on the incidence of disease has been clearly brought out and correlated with the cultural behaviour of the parasitic fungi.

4. Methods of healthy propagation of young suckers as well as curing fruits free from disease have been dealt with.

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STUDIES ON *PENNISETUM TYPHOIDEUM* (RICH.)—THE PEARL MILLET, PART I.

ANTHESIS.

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(With Plate LIV and one text-figure)

The commonly cultivated pearl millet has had more systematic names probably than any other grass. Early in the last century it had almost as many names as there were floras. *Pennisetum typhoideum, Penicillaria spicata, Penicum spicatum, Pennisetum alopecuroides,* were the most common. By the middle of the last century the other names had mostly dropped out of use, giving place to *Pennisetum typhoideum* [Chase, 1921].

Among the common English names are, pearl millet, bulrush millet, and cat-tail millet. In Europe it goes by the name of candle millet and dark millet. In India the most common names are, *bajra* and *bajri* (N. India), *cumbu* (Tamil), and *sajja* (Telugu).

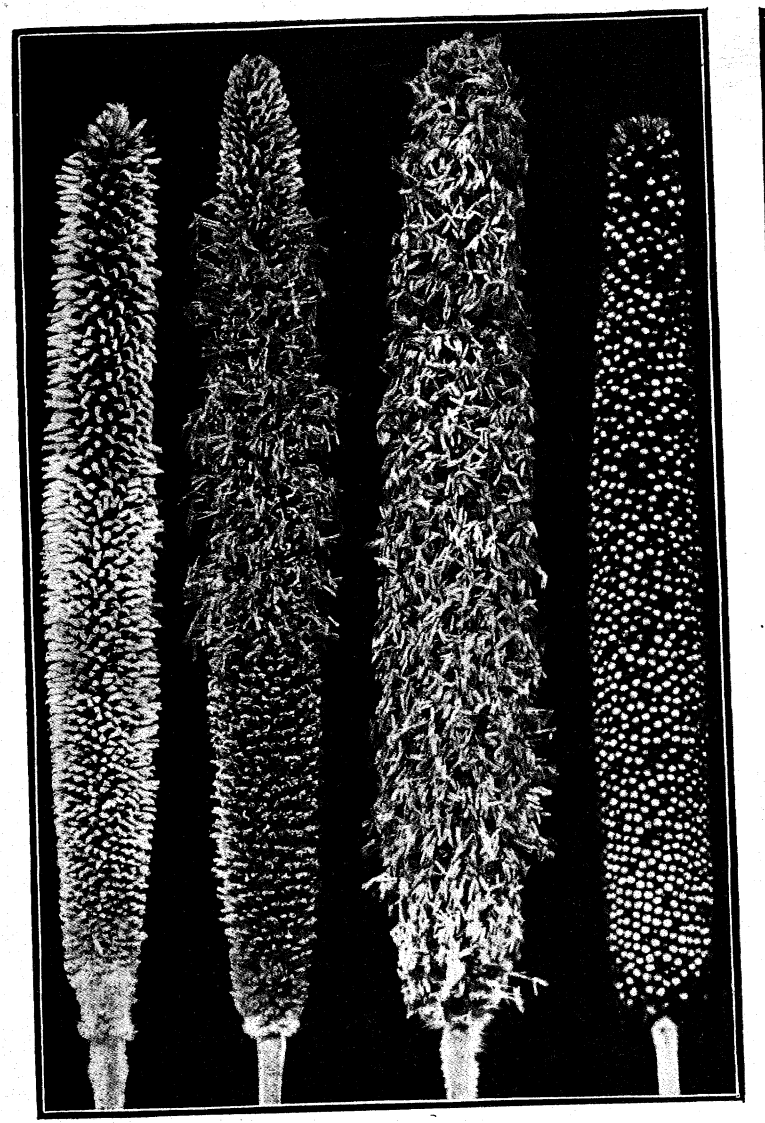
Economic aspects of anthesis study.

The study of anthesis in millets was one of the first activities of the Millet Breeding Station, Coimbatore. *Pennisetum typhoideum* was singled out for this initial work because in this millet with its mass of cylindrical heads, the anthers are so very obtrusive in their existence and persistence, that they easily attracted attention (Plate LIV).

In the course of enquiries made during seed collection time, the fact was brought to notice that there was a belief among cultivators that rain at flowering time meant failure of the crop and in the district of Vizagapatam the belief was prevalent that a good mid-night downpour spelled disaster to the crop. An attempt was made to ascertain the cause of this belief.

Previous work.

The anthesis in this millet has attracted the attention of many earlier workers [Kornicke, 1885; Leeke, 1907 (quoted by Fruwirth); Robinson, 1917; Fruwirth,



(a) (b) (c) (d)

Fig. 1.—(a) Protogyny. (b) First anthers. (c) Felt of persistent dry anthers. (d) Set grains.

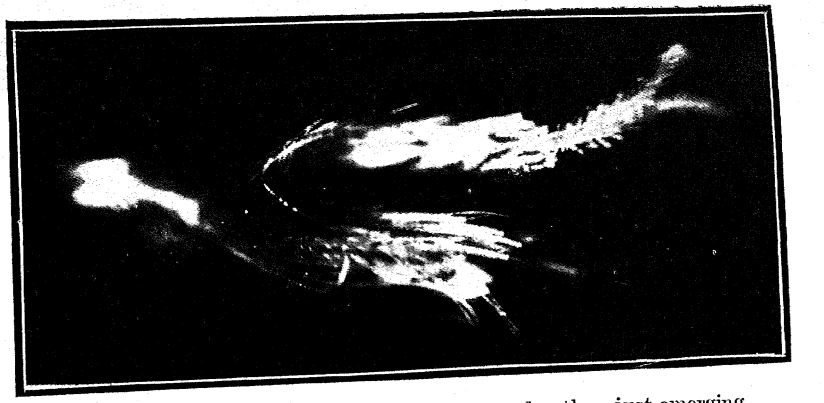


Fig. 2.—Hermaphrodite flower with stigma and anthers just emerging.

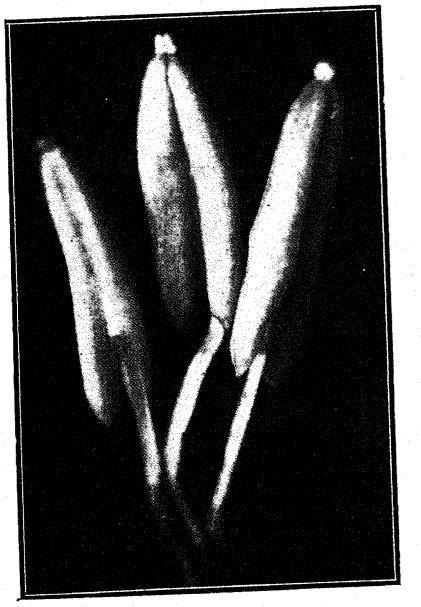


Fig. 3.—Penicillate anthers.

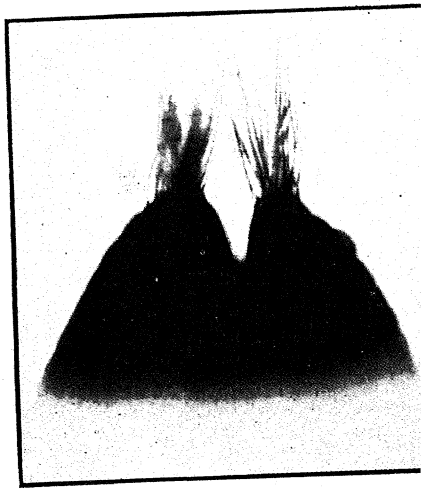
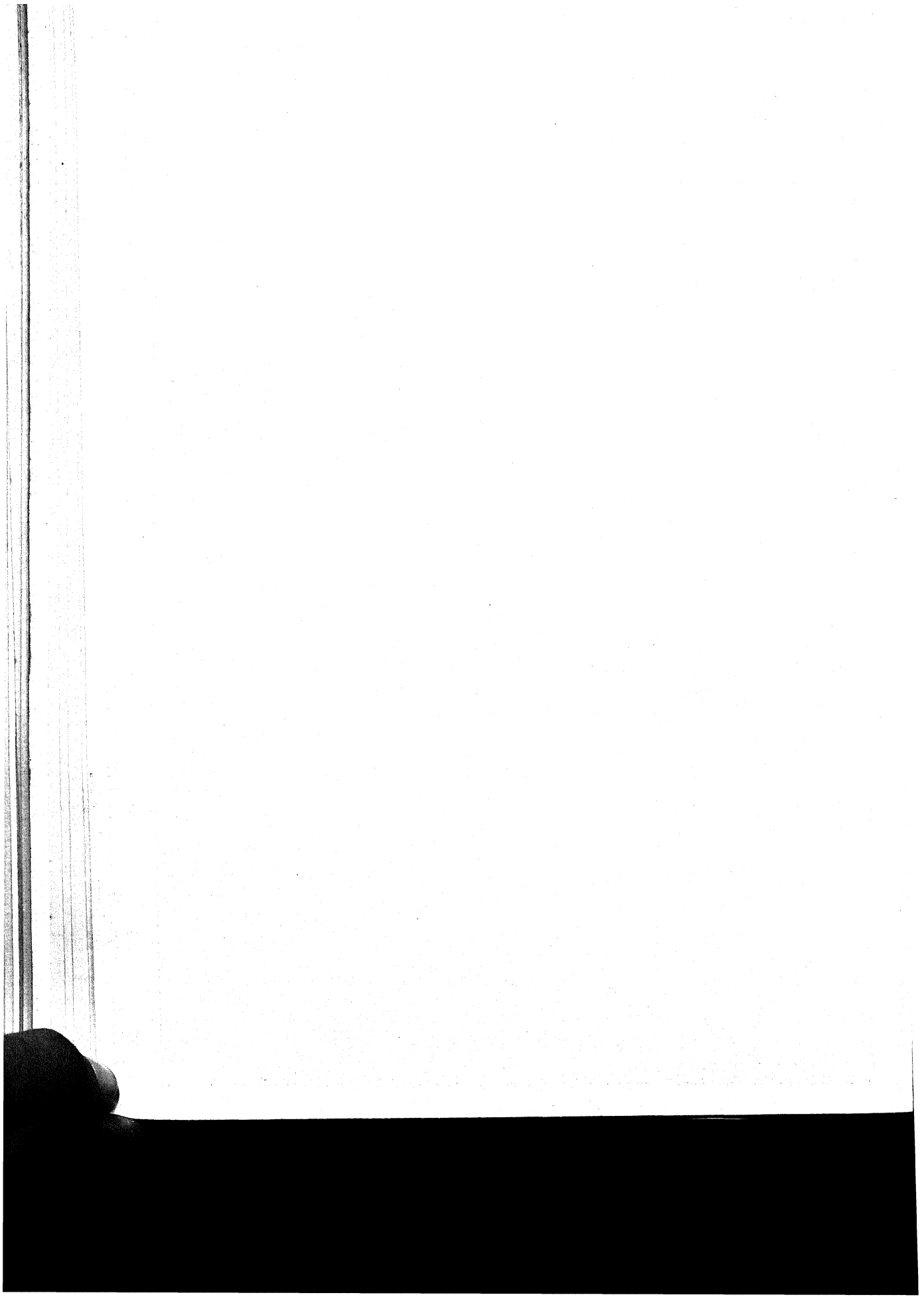


Fig. 4.—Pencils enlarged.



1923; Godbole, 1927; and Patwardhan, 1927]. Neither author, however, has given a detailed record of observations on a flowering head carried throughout the day and night.

Inflorescence.

The inflorescence of this millet is a compound spike with an unbranched tapering central axis. On this axis a number of rachillæ run obliquely disposed in series like the cells of a honey-comb. Ordinarily each rachilla carries two spikelets enclosed in a whorl of bristles. Rachillæ bearing one or three spikelets also occur while those bearing four are rare.

After an examination of a number of earheads we can say that about three-fourths of the rachillæ are two-spikeletted, that nearly a fourth are single-spikeletted, and that a negligible number may be three, four, or very rarely five-spikeletted. There is a tendency for the single-spikeletted rachillæ to be present in larger numbers towards the tip of the earhead, and for the three and four-spikeletted ones to occur more frequently at the bottom.

Protogyny.

The most noticeable feature of the earhead on emergence is its protogynous condition with a mass of protruding, glistening stigmas (Plate LIV, fig. 1). The stigmas take a long time to push their way out of the glumes. The time taken varies from 12 to 24 hours according to the season. The forking of the stigmas occurs only after its full growth. Stigmas remain fresh from 12 to 24 hours according to weather conditions.

Typical anthesis of a flower.

The emergence of the anther from the flower in this lodiculeless millet was observed in detail. It was noticed that from the emergence of the tip of the anther to the completion of its dehiscence it took about an hour in the morning and about twice that time at mid-night. In the day time the actual pushing out of the anthers from the glumes takes about half an hour, the growth of the comparatively stiff filament about a quarter of an hour, and the process of the dehiscence about twenty-five minutes. At night the pushing out of the anther takes well over an hour, with slight increases in the time taken for the elongation of the filament and the dehiscence of the anthers. The protruding stigmas lead the way and the penicillate anthers seem to be a special adaptation for this slow and laboured emergence of the anthers, lacking lodicule facilities for glume opening (Plate LIV, fig. 2). An examination of a number of lodiculeless grasses shows in many cases that they possess pointed, mucronate, apiculate or penicillate anthers and it would seem therefore that the pencils of this "Penicillaria" perform a definite function in aiding the emergence.

Anther flushes.

There are two distinct waves of anthesis, the first being the anthesis of the bisexual flowers which commences even before the complete protrusion of the stigmas in the lower part of the earhead. This first wave may begin from two to three days after the protrusion of the first stigmas in the earhead. This flush works its way down the head and before it is complete, and concurrently with the final stages of its progress, the second flush of staminate flowers commences. A number of observations in isolated spikelets in different seasons in the various parts of the earhead have been made, and point to the second flush beginning a day to a day-and-a-half after the commencement of the first flush. The progress of the stigma protrusion, the first flush of anthers from bisexual flowers, and the second flush of anthers from unisexual flowers, may each be set down to run about the same periods, with partial overlappings, so that in our experience no definite breaks have been noticed between the two flushes of anther protrusions [Burns and Barve, 1932].

PRELIMINARY STUDIES.

Preliminary observations on the march of anthesis on an earhead were made in the year 1922 and left no doubt whatever as to the fact that in this millet anthesis was going on throughout the day and night with a concentration at midnight. [Rangaswami Ayyangar, 1923].

Further intensive work was obviously needed and was attempted under artificial conditions. Three earheads were cut and kept in water inside the laboratory. Anthesis proceeded as usual but stopped at the end of two and a half days. The earhead in Crone's solution fared no better. Periodical records revealed the slackening of anthesis about 4 p.m., and a concentration between 10 p.m. and 12 midnight—a repetition of the out-door habit. An analysis of the earheads at the cessation of anthesis showed that in the complete flowers about four per cent. did not exert their anthers and that in the purely staminate flowers, there was only a partial protrusion. There was thus no use continuing the observations on cut heads to have a detailed record of complete anthesis.

Observations continued in the field and the results of these studies were presented in a paper at the Indian Science Congress [Rangaswami Ayyangar, 1924].

These observations were made on *kattu kumbu*, the dry-land variety of the Coimbatore district; the method of recording was every four hours both day and night, the two recorders taking turns. Each set of three anthers that was exerted was taken for one floral unit and the counts thus made. The head under observation took eight days to flower. The second day recorded the largest number of opening flowers. In the first three days more than half the number of flowers

had opened. Flowering proceeded steadily from top to bottom throughout the flowering period. The following are the records at the hours noted below :—

6 a.m.	338
10 a.m.	327
2 p.m.	158
6 p.m.	42
10 p.m.	303
2 a.m.	684

The increasing work on sorghum and *ragi* made a detailed pursuit of the phenomenon impossible until the year 1928 when the observations were continued during the main season. Three contemporary heads of the same *kattu kumbu* were under observation and the records of their continued anthesis are given below :—

6 a.m.	2296
10 a.m.	1513
2 p.m.	468
6 p.m.	206
10 p.m.	898
2 a.m.	3035

There is thus a close parallel with the results obtained in 1922.

Detailed studies.

Very detailed observations were made in the summer of 1929 on the same local variety. Records were taken at two-hour intervals both day and night on four contemporary earheads. The results are tabulated below :—

Summer 1929—kattu kumbu—*17th to 26th June 1929.*

——	Head 1	Head 2	Head 3	Head 4	Total
6 a.m.	178	96	159	165	598
8 a.m.	107	131	166	135	539
10 a.m.	135	135	215	250	735
12 noon	113	75	129	163	480
2 p.m.	43	63	89	115	310
4 p.m.	42	55	65	93	255
6 p.m.	57	116	83	169	425
8 p.m.	101	379	194	448	1122
10 p.m.	231	439	329	576	1575
12 midnight	304	285	333	387	1309
2 a.m.	295	166	319	346	1126
4 a.m.	223	86	160	237	706
	1829	2026	2241	3084	9180

The above data leave little doubt that the pearl millet flowers throughout the day and night. It will be noticed that the heaviest anthesis is in the period prior to midnight and that the lowest is in the afternoon towards 4 P.M., following the period of heavy transpiration. The tendency towards a slight rise prior to 10 A.M. in the forenoon may possibly mark the recuperative effects of sunrise.

The march of anthesis together with the march of temperature and humidity is charted in the accompanying graph (Fig. 1).

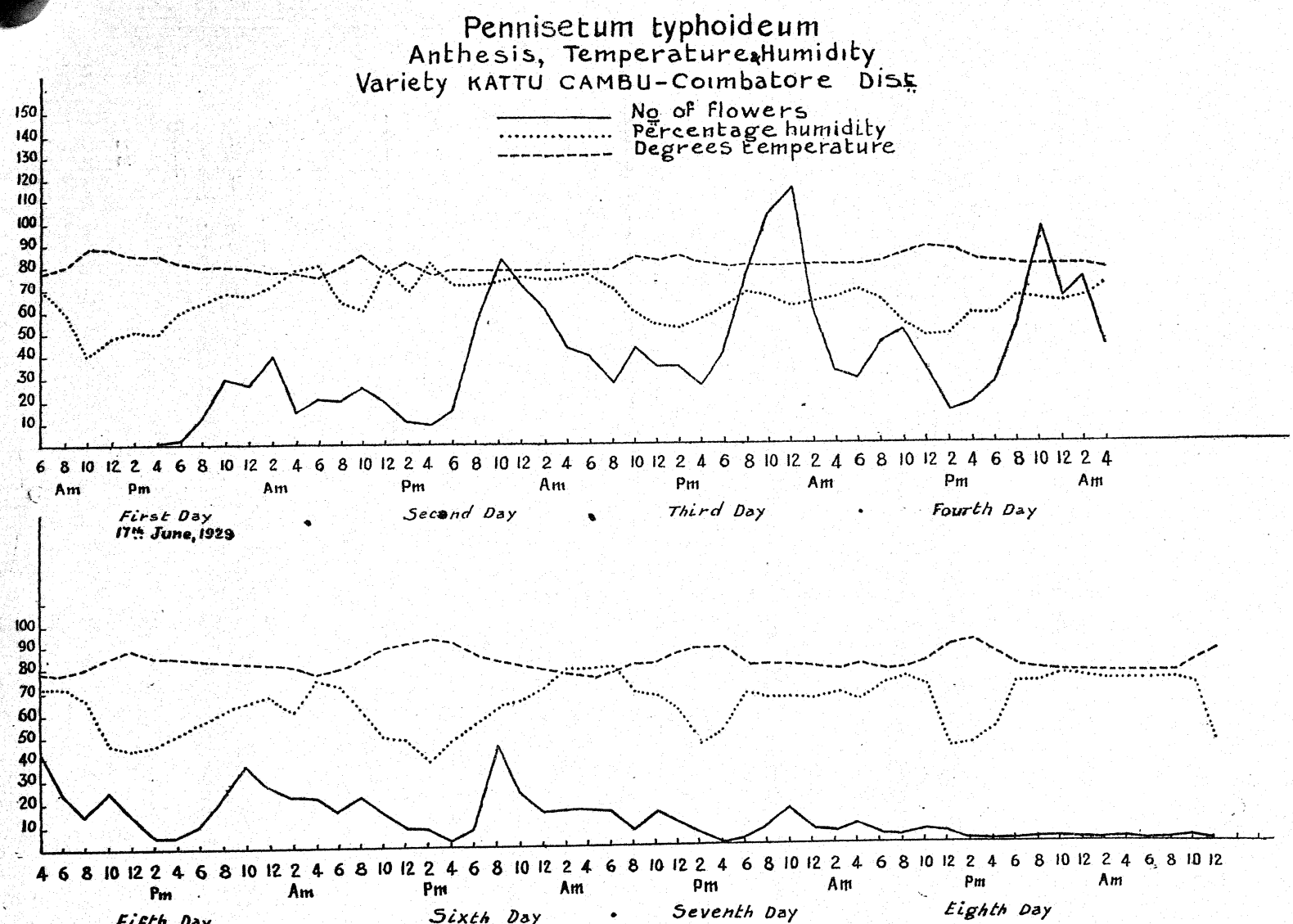

Pennisetum typhoideum
Anthesis, Temperature & Humidity
Variety KATTU CAMBU-Coimbatore Dist.
No of Flowers
Percentage humidity
Degrees Temperature
First Day
17th June, 1929
Second Day
Third Day
Fourth Day
Fifth Day
Sixth Day
Seventh Day
Eighth Day
Am
Pm

Fig. 1.

It will be noticed generally that there is a tendency for periods with an increase in humidity and a decrease in temperature to mark periods of great activity in anthesis, there being a corresponding lessening with a decrease in humidity and an increase in temperature. The maximum flush is ahead of periods of low temperature and high humidity. The response of the plant to the beginnings of these changes is quick and leads to the optimum conditions favouring anthesis, resulting in a rapid flush to be followed by a gradual slackening, marking the exhaustion due to this flush of anthesis.

Discussion.

The Italian millet flowers practically all the 24 hours except that there could be said to be a break between 10 a.m. and 8 p.m. barring odd flowers. The pearl millet is therefore the only millet in which there is definite anthesis, though of varying degrees, all the hours of the day and night. Unlike the Italian millet there is not the packing in mass of flowers even in the interior of the earhead. The crowding of the flowers in one plane is possibly a feature of this millet. The anther flowers add to this crowding, so much so, that it looks likely that this crowding is one of the main factors responsible for this continued flowering. The absence of lodicules, while it may connote an absence of regulation, cannot be the primary determinant in this peculiar floral conduct. The Italian millet with its lodicules closely imitates the pearl millet. Moreover a study of the wild cousins of the pearl millet, with lax earheads, shows that the absence of lodicules notwithstanding, they are not continuous in their flowering. For instance, *P. rupellii* flowers from about 11 p.m. to 11 a.m., *P. cenchroides* from 3 a.m. to 11 a.m. and *P. purpureum* from 7 a.m. to 9 p.m. It therefore looks as if a study of definitely lax-spiked varieties of this millet may throw light on this phenomenon of ceaseless flowering. On the whole the broad fact remains that in this millet we have a plant whose anthesis will afford a live measure of the various physiological and metabolical processes produced in the plant through environmental changes. It is suggested that this common millet may figure a little more prominently in the academic institutions for plant study.

Summary.

Anthesis in the pearl millet goe son throughout the day and night. Two-hour observations on a number of earheads in three seasons show that the maximum flowering is between 10 p.m. and 12 midnight. During the day there is a second slight rise prior to 10 a.m. At 4 p.m. the weakest flowering is met with.

Periods of high humidity and falling temperature are marked by increased anthesis.

The flush of anthesis in the staminate flowers overlaps the first flush of anthesis in the hermaphrodite flowers.

Individual flowers take about an hour for the exertion and opening of the anthers by day time. At night the time is about doubled. The absence of lodicules disfavours rapid and concentrated anthesis and the penicillate anthers seem an adaptation for anther protrusion.

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ABSTRACT

Cumin powdery mildew in Bombay. B. N. Uppal and M. K. Desai. (*Dept. Agric., Bombay Bul.* **169**, 1-16 ; 1933).

Powdery mildew appears first on the lower leaves as grayish specks, which rapidly enlarge and cover the filiform leaves with a thin, powdery growth. Under favourable conditions infection spreads rapidly to flowers and fruits, on which the fungus makes a dense growth.

The fungus develops with extreme rapidity at temperatures between 80 and 95°F. Temperatures below 80°F. are unfavourable for mildew.

Proof of the perennation of the organism as dormant mycelium on the seed has been established experimentally.

The morphology of the organism has been fully described.

The haustoria of the fungus are lobed, and the conidia are cylindric and flat-ended. Because of these two characters and the size of conidia, the organism is referred to the species *Erysiphe polygoni* D C.

Cumin mildew can be controlled by one application of sulphur made at about the time of flowering, and the quantity of sulphur required per acre is 25 lbs. (B. N. U.)

NOTES

AGRICULTURAL PARASITOLOGY—HELMINTHOLOGY.

In *Agriculture and Livestock in India* for July 1933 there appears a paper by Dr. Peters on the scope and aims of the Imperial Bureau of Agricultural Parasitology which was read at the British Association Symposium on Applied Helminthology. The subject-matter of the Bureau's work comprises such helminthological data as may be of importance to agriculture in its widest sense. Though specialised and restricted so far as the zoological status of the parasites is concerned, the work of the Bureau has exceptionally wide contacts with the various branches of pure and applied science. Of special interest to research workers in crop production and soil science are the following publications of the Bureau :—

1. The Root-infesting Eelworms of the Genus Heterodera—(Bibliography and Host list.) Price 6*s*.
2. The Eelworm *Heterodera schachtii* as a Potential Danger to the Sugar-beet Industry. Notes and Memoranda No. 1—Price 1*s*.
3. Differential Diagnosis of Plant-parasitic Eelworms—Notes and Memoranda No. 5 (1932)—Price 1*s*.
4. Potato Sickness and the Eelworm *Heterodera schachtii*—Notes and Memoranda No. 6 (1932)—Price 2*s*.

The Animal Husbandry Expert to the Imperial Council of Agricultural Research is the official correspondent for this Imperial Bureau and will be glad to furnish fuller information or to receive suggestions.

BIBLIOGRAPHY OF TROPICAL AGRICULTURE.

The International Institute of Agriculture, Rome, has recently issued a Bibliography of Tropical Agriculture, 1931, in English, price ten liras per copy. It is valuable to Indian workers as a useful supplement to existing abstracting journals, and at ten liras the book is not expensive. The Institute has now resumed direct sale of its publications and copies can be purchased direct from the Secretary-General, International Institute of Agriculture, Rome, or through the usual booksellers.

BIBLIOGRAPHY ON THE BREEDING AND GENETICS OF THE MILLETS AND SORGHUMS.

(Imperial Bureau of Plant Genetics, School of Agriculture, Cambridge, Price 1*s*.)

The Bibliography is of special interest to botanists in India not only because much of the work quoted has been done in India but on account of the great

importance of the millets to Indian agriculture. The crosses between sugarcane and sorghum made at Coimbatore by Venkataraman and Thomas and between Kaffir corn and sugarcane in Java further add to the economic interest of this important group of crops.

THE INTERNATIONAL YEARBOOK OF AGRICULTURAL STATISTICS.

The International Institute of Agriculture at Rome has recently published the 1931-32 edition of the "International Yearbook of Agricultural Statistics".

This volume of about 800 pages is the result of the most extensive and detailed inquiry made in the domain of international agricultural statistics and constitutes a work of the greatest importance to all those who are interested in questions having a direct or indirect relation to production and commerce of agricultural products.

In the first part of the Yearbook are classified the figures for area and population in the years nearest to 1927 and 1931 for 208 countries: the presentation of these figures throws light upon the world situation from the geographical, political and demographical points of view during the post-war period. The second part is composed of a series of tables comprising for nearly 50 countries the available data concerning the uses for which the total area is employed, the apportionment of cultivated areas between the different crops, agricultural production, numbers of the different kinds of livestock and the products derived from them. In the tables constituting the third part of the volume, have been indicated for nearly 40 agricultural products, the area, production and yield per acre in each country during the five years, 1923-27, and during each of the years from 1928 to 1931.

For each kind of livestock all available figures in the different countries have been grouped for the years 1927 to 1931. A large part of the volume is devoted to statistics of the commercial movement of 43 vegetable products and 13 products of animal origin. The figures published relate to the imports and exports during the calendar years and for the cereals also during the commercial seasons.

It may be added that the tables of production and commerce not only specify details for each country but also the totals for the different continents and hemispheres and for the whole world, allowing the formation of a general idea of the changes taking place during the periods under consideration in the area under each crop, quantities harvested and the commercial movement in each product.

The part devoted to prices contains the weekly quotations of 25 agricultural products on the principal world markets for the period January 1927 to July 1932. In the freights section will be found the quotations for the transport of wheat, maize and rice on the most important shipping routes, and in the section reserved for fertilizers and chemical products useful in agriculture are published statistics of

production, trade, consumption and prices for 15 products. In the Appendix have been brought together special chapters on the distribution of agricultural holdings according to their size and mode of tenure. The Forestry Statistics have been extended and developed and will be published in a separate volume under the title of 'International Yearbook of Forestry Statistics'.

THE DISPERSION OF SOILS IN MECHANICAL ANALYSIS.

(Imperial Bureau of Soil Science Technical Communication No. 26.)

In view of the importance of mechanical analysis in the examination of soils and the desirability of securing uniform and convenient methods, applicable as far as possible to all soils and all climates, an investigation of methods of dispersion was undertaken at the instance of the Imperial Bureau of Soil Science by Professor G. W. Robinson of the University of North Wales, Bangor*; the work was financed throughout by the Empire Marketing Board.

The results of Professor Robinson's investigations are described in this Technical Communication. Most of the soils he examined were obtained through correspondents of the Bureau, and it will be seen that they include a very wide selection of the soil types found within the British Empire.

Comparability of analytical results is a first essential to securing that co-operation in imperial research which it is the Bureau's aim to foster. If research workers throughout the Empire, as far as possible, adopt Professor Robinson's recommendations, it would be a first step towards a greater degree of uniformity in all analytical procedure. The recommendations should not be considered as necessarily final, and any criticisms of them, or reports of difficulties encountered in carrying them out, will be welcomed by the Imperial Bureau of Soil Science.

WOOD HOUSE MEMORIAL PRIZE, 1933.

In memory of Mr. E. J. Woodhouse, late Economic Botanist and Principal of Sabour Agricultural College who was killed in action in France in 1917, a prize in the form of a silver medal and books of a combined value of Rs. 85 will be awarded to the writer of the best essay on a subject of botanical interest to be selected from the list noted below. The length of the essay should not exceed 4,000 words.

The competition is open to graduates of Indian Universities and to Diploma holders and Licentiates of recognised Agricultural Colleges in India who are not more than 30 years of age on the date of submission of their essays.

* The analytical work was carried out by Dr. M. Richardson, at Bangor.

Papers should be forwarded to the Director of Agriculture, Bihar and Orissa, Patna, before November 1st, 1933.

Failing papers of sufficient merit no award will be made. Essays must be typewritten and on one side of paper only.

List of subjects for 1933 prize :—

1. The root system as a limiting factor in plant distribution.
2. Basis of quick selection for drought resistance and water logging in agricultural crops.
3. Application of modern statistical methods to yield trials.

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Influence of Manures on the Wilt Disease of *Cajanus Indicus* Spreng and the Isolation of types Resistant to the Disease.

BY

W. McRAE, M.A., D.Sc. (EDIN.), F.L.S.,
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Imperial Economic Botonist, Imperial Institute of Agricultural Research, Pusa.

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ORIGINAL ARTICLES

OBSERVATIONS ON THE LIFE-HISTORY, BIONOMICS AND CONTROL OF THE WHITE-FLY OF COTTON (*BEMISIA GOSSYPIPERDA* M. & L.).

BY

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AND

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(Received for publication on 22nd October 1932.)

(With Plates LV-LX and five text-figures.)

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FOREWORD.

Some of the observations made by the junior author during 1929-30, at Khanewal, and given in this paper, have been included by Mr. R. Thomas in his

article "Periodic Failure of the Punjab-American Cotton Crop", published in the *Agriculture and Live-stock in India* (II, May, 1932). We desire to make it clear that we do not agree with his conclusions.

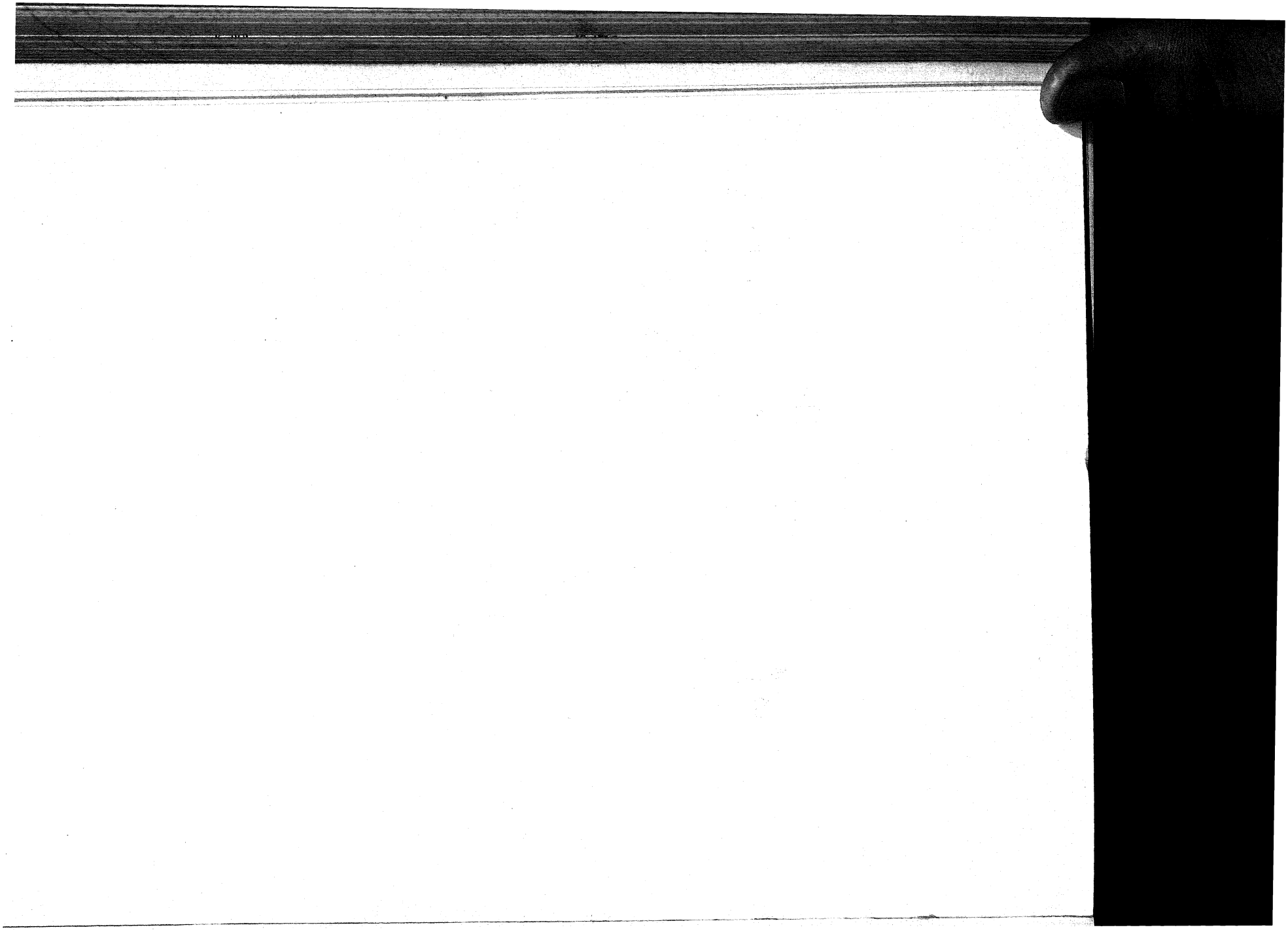
M. AFZAL HUSAIN.
KIDAR NATH TREHAN.

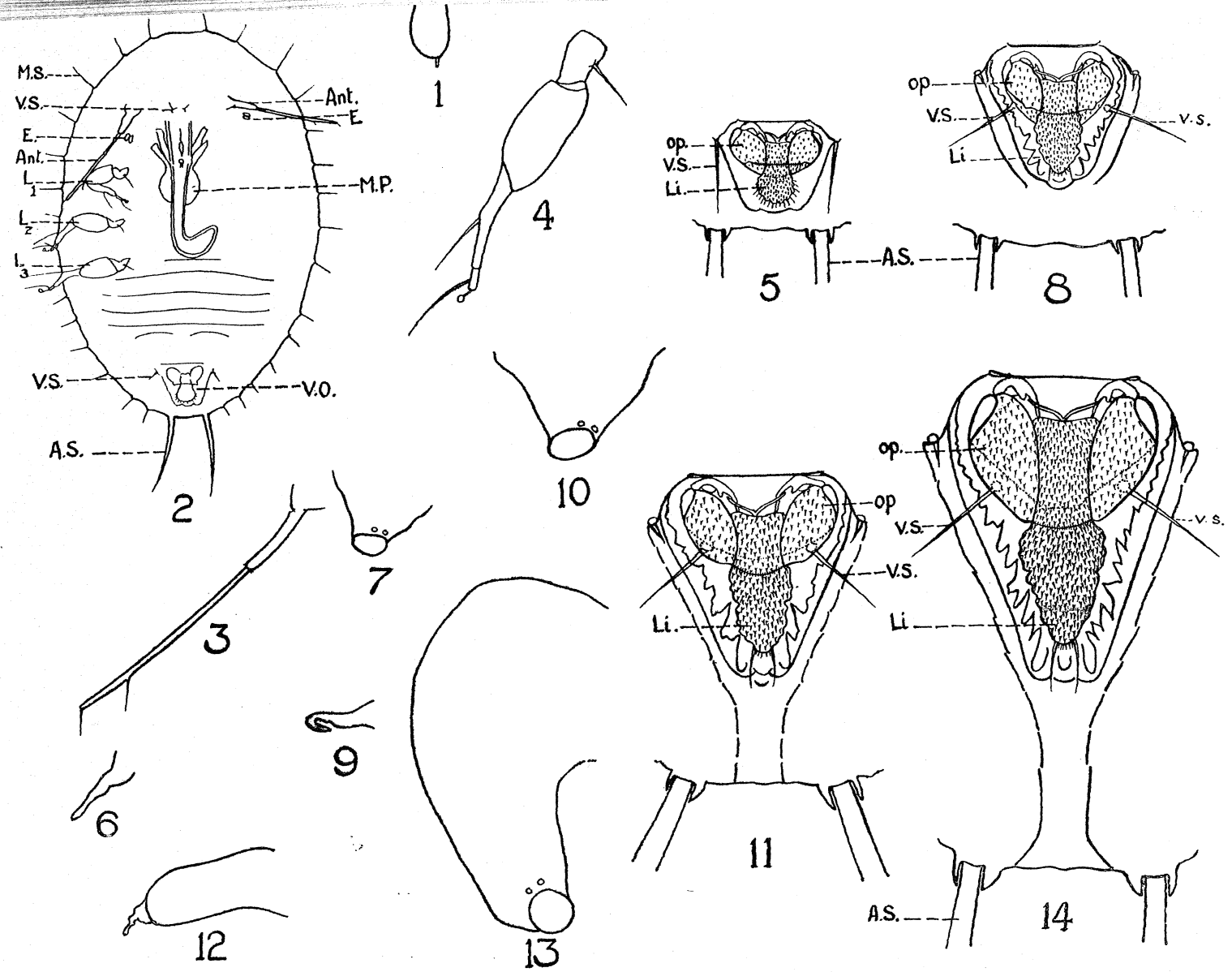
INTRODUCTORY.

The so-called 'Punjab-American' varieties of cotton, grown in the Punjab, have occasionally failed, more or less badly, over extensive tracts. The most serious general failures occurred during 1919 and 1926, and 1921, 1927 and 1928 were the years of partial failure. These failures have drawn a great deal of attention [Milne, 1922, 1928; Roberts, 1929, 1930; Trought, 1931]. The general symptoms of a failing crop have been stated as: stunted growth, yellowing and reddening of leaves, premature defoliation, softness of young bolls, bad opening and poorly developed lint and seed [Milne,1922; Roberts, 1929; Afzal Husain, 1930; and Trought, 1931]. Milne [1928] explains this failure by something comparable to 'heat stroke', while Trought [1931] considers the influence of adverse environmental factors on root and shoot development as the real cause. In 1928, Mr. Roger Thomas* of the British Cotton Growing Association, Khanewal (Punjab), put forth a suggestion, that it was the White-fly which was mainly responsible for the failure of the 1928 crop [Roberts, 1929]. Roberts [1930] supporting this view went a step further and asserted that all the previous failures of the 'American' varieties of cotton were caused by this insect, although this cause remained unknown at the time. Misra and Lamba [1929] have also accepted the White-fly theory of the cotton failures. This question has already been very fully discussed by Afzal Husain [1930]. *B. gossypiperda* has, therefore, come into prominence as a serious pest of cottons and as a possible cause of the cotton failures.

Casual observations on this insect were started as early as 1922, but systematic investigations were started by the Department of Agriculture, Punjab, in 1928. The Indian Central Cotton Committee helped this undertaking by awarding in 1929, a senior research studentship to the junior author. Most of the observations mentioned in this paper were made at the farm of the British Cotton Growing Association, Khanewal, and some at the Agricultural College, Lyallpur. The facilities provided by the British Cotton Growing Association, the personal interest taken by Messrs. Roberts and Roger Thomas, and the help given by S. Datar Singh, Manager of the Farm, are gratefully acknowledged.

*Mr. Roger Thomas has fully discussed his views in his article Periodic Failure of the Punjab-American Cotton Crop—*Agriculture and Live-stock in India*, Vol. II, Part III, May, 1932.




M.S.
V.S.
E.
Ant.
L1
L2
L3
Ant.
E
M.P.
V.S.
V.O.
A.S.
2
1
4
op.
V.S.
Li.
5
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V.S.
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A.S.
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13
op.
op
V.S.
V.S.
Li.
11
op.
V.S.
V.S.
Li
A.S.
14

PLATE LV.

WHITE-FLY OF COTTON (*Bemisia gossypiperda*).

Fig. No.

1. Egg . . . . . × 75
2. Nymph—1st instar . . . . . × 265
3. Antenna of 1st instar nymph . . . . . × 712
4. Leg of 1st instar nymph . . . . . × 712
5. Vasiform orifice of 1st instar nymph . . . . . × 675
6. Antenna of 2nd instar nymph . . . . . × 637
7. Leg of 2nd instar nymph . . . . . × 637
8. Vasiform orifice of 2nd instar nymph . . . . . × 675
9. Antenna of 3rd instar nymph . . . . . × 637
10. Leg of 3rd instar nymph . . . . . × 637
11. Vasiform orifice of 3rd instar nymph . . . . . × 675
12. Antenna of pupa . . . . . × 525
13. Leg of pupa . . . . . × 525
14. Vasiform orifice of pupa . . . . . × 675

Abbreviations in the figures.

Ant.	Antenna.	Li	Lingula.
A. S.	Anal spine.	M. P.	Mouth parts.
E.	Eye.	M. S.	Marginal spine.
L_1	Front leg.	Op.	Operculum.
L_2	Middle leg.	V. O.	Vasiform orifice.
L_3	Hind leg.	V. S.	Ventral spine.

PLATE LV.

White-fly of cotton (Dialeurodes gossypiperda).

Fig. No.		
1.	Egg	× 75
2.	Nymph—1st instar	× 205
3.	Antenna of 1st instar nymph	× 712
4.	Leg of 1st instar nymph	× 712
5.	Vasiform orifice of 1st instar nymph	× 675
6.	Antenna of 2nd instar nymph	× 637
7.	Leg of 2nd instar nymph	× 637
8.	Vasiform orifice of 2nd instar nymph	× 675
9.	Antenna of 3rd instar nymph	× 637
10.	Leg of 3rd instar nymph	× 637
11.	Vasiform orifice of 3rd instar nymph	× 675
12.	Antenna of pupa	× 285
13.	Leg of pupa	× 285
14.	Vasiform orifice of pupa	× 675

Abbreviations in the figures.

Ant.	Antenna.	Li	Lingula—
A. S.	Anal spine.	M. P.	Mouth parts.
E.	Eye.	M. S.	Marginal spine.
L_1	Front leg.	Op.	Operculum.
L_2	Middle leg.	V. O.	Vasiform orifice.
L_3	Hind leg.	V. S.	Ventral spine.

Distribution.

B. gossypiperda is widely distributed in the Punjab and has been recorded from cottons from almost all the districts of the Province. The severity of attack is, however, confined to Lyallpur, Jhang, Shahpur (Sargodha), Multan and Montgomery district—the so-called 'canal colony tract'.

The presence of *B. gossypiperda* on cotton at Pusa (Bihar and Orissa) was recorded, as early as 1905 [Misra and Lamba, 1929]. So far no information is available regarding the occurrence of this insect in other parts of India.

Kirkpatrick [1930] mentions the same species from the Sudan, as causing leaf-crinkle. An un-identified aleurodid is mentioned by Golding [1930]* as causing leaf-curl in South Nigeria. A further study of the distribution of this insect would be most useful.

If the species found in India, the Sudan, South Nigeria and Iraq is *B. gossypiperda* M. and L., its simultaneous appearance as a major pest in widely separated parts of the globe is not without significance. It may be an instance of a species at a stage in its evolution when it is showing both morphological and physiological plasticity and is undergoing striking mutations. The fluctuations exhibited by *B. gossypiperda* in the number of the dorsal spines in the pupal stage, may be an index of its morphological variation. It appears that its polyphagous habit is also a recent sudden development and may reflect an abrupt change in its host selection and feeding habits, and, therefore, in its physiology.

Description of the various stages.

Misra and Lamba [1929] have described the various stages of this insect, but as their descriptions are generally not quite satisfactory and are in parts wrong, it has been considered advisable to give detailed descriptions of all the stages.

Egg (*Plate LV, fig. 1*).—Sub-elliptical, apical end narrower, pedunculate, the stalk is inserted into the tissue of the leaf, chorion smooth, colour light yellow when fresh, subsequently changing to dark brown, the distal free end is of deeper colour. Length 0·168-0·176 mm., breadth 0·08-0·09 mm., average measurements 0·171 × 0·082 mm., stalk 0·016-0·024 mm., long, average 0·019 mm.; reddish eye spots conspicuous before hatching and distinctly divided.

Nymph of 1st instar (*Plate LV, fig. 2*).—Elliptical, light yellow. Length 0·248-0·256 mm., breadth 0·136-0·144 mm., average measurements when newly hatched 0·253 × 0·139 mm., margin surrounded by waxy bands and beset with 16 pairs

*Roger Thomas [1932] also mentions Iraq as one of the localities where a cotton White-fly is present as a minor pest. An aleurodid has also been mentioned from cottons in China in 1805 [Roberts, 1929].

of bristles, three cephalic—the last cephalic pair being the longest, ten laterals—sub-equal, three caudals—the anal pair being the longest. Two pairs of ventral spines—the anterior pair in front of the rostrum and the posterior pair at the level of the dorsal vasiform orifice. The dorsal spines which are present in the later stages are absent. Antennae thin and long, 0·066-0·073 mm. in length, average 0·070 mm., three segmented—basal segment thick, 2nd cylindrical and the 3rd segment elongated and provided with two spines—one at a distance of $\frac{2}{3}$ of the length of the segment from the base and the other at the tip (Plate LV, fig. 3). Eyes distinctly divided. Legs functional, measuring 0·07 mm. and reaching beyond the margin of the body when extended; tibia armed with a long curved bristle, tarsus single-jointed with a bristle near the distal end, longer than the tarsus; pedunculated disc like pad at the end of the tarsus (Plate LV, fig. 4). Mycetoma orange yellow. Vasiform orifice slightly wider than long (Plate LV, fig. 5), length 0·020-0·028 mm., breadth 0·024-0·030 mm., average measurements 0·024×0·027 mm.; operculum semicircular; lingula setose, twice as long as the operculum and, therefore, projecting beyond it.

Nymph of 2nd instar.—Body elliptical, depressed, pale-greenish-yellow in colour. Length 0·312-0·350 mm., breadth 0·192-0·200 mm., average measurements 0·319×0·196 mm. Margin crenulate with a waxy band all round. Eyes divided, antennae* much shorter than in the previous stage, directed backwards (Plate LV, fig. 6), 0·021 mm. long. Legs degenerate (Plate LV, fig. 7), unsegmented, conical stumps, each ending in a disc like sucker. Mycetoma orange-yellow. Vasiform orifice (Plate LV, fig. 8) measuring 0·030×0·032 mm., lingula armed with a pair of long hairs at the tip. Dorsal bristles absent or 1-3 pairs, marginal bristles only one pair of anals.

Nymph of 3rd instar.—Shape corresponding to the previous instars, length 0·48-0·60 mm., breadth 0·32-0·36 mm., average measurements 0·52×0·34 mm. Eyes not completely divided; antennae directed towards the median line, hook-shaped, the convexity of the hook antero-medium, 0·020 mm. long (Plate LV, fig. 9). Legs (Plate LV, fig. 10) as in the preceding instar. Vasiform orifice (Plate LV, fig. 11) 0·051×0·48 mm. Dorsal spines and mycetoma as in the 2nd instar.

Pupa.—Body slightly convex, 0·6×0·4 mm. to 0·8×0·6 mm., colour deep yellow, eyes well developed, not completely divided. Antennae better developed than those in the 2nd and 3rd instars (Plate LV, fig. 12), 0·056 mm. long, crenulate, thick and pointing backwards and outwards, each terminates in a slightly bent thin process. Legs curved, unsegmented (Plate LV, fig. 13). Vasiform orifice longer than broad (Plate LV, fig. 14), length 0·066-0·083 mm., breadth 0·050-0·058 mm., average

* Misra and Lamba [1929] incorrectly state that the antennae are absent in the 2nd instar nymph. They are present in all nymphal stages.

measurements 0·077×0·052 mm. Dorsal spines variable (see below), when a full complement of seven pairs of dorsal spines is present, the arrangement is as follows :—

I. Cephalic, preocular ; II. post ocular ; III. lateral, at the level of the middle legs ; IV. lateral just behind the above ; V. dorso-medial ; VI. postero lateral ; on the sides of 3-4 abdominal segments ; VII. vasiformal on the side of the vasiform orifice. Besides these, there is a pair which is anal.

Variations in the distribution of dorsal spines in B. gossypiperda.—Except in the 1st instar the number and distribution of the dorsal spines is very variable. As the pupal stage is used in identification, and the number and arrangement of spines is one of the recognized constant characters, these variations are of great significance. Misra and Lamba [1929] overlooked these. Almost all the combinations from a full complement of seven pairs of spines to their total absence, are met with. This variation may even be found in the progeny of the same pair and occurs in both sexes.

Adult (*female*).—Length 1·1-1·2 mm. ; body yellow with two pairs of concolourous wings, eyes constricted in the middle forming two parts, lower facets bigger than the upper. Antennae seven segmented, elongated (for measurements see table below)—Segment I. sub-globose, II. sub-pyriform, III. elongated, longest of all and beset with sensoria distally, IV. cylindrical, V. clavate, VI. cylindrical, VII. elongated and imbricate, provided with sensoria and terminating in a spine. Labium 0·39 mm. long, fore-wing measuring on an average 0·89×0·34 mm., venation poorly developed—radial sector and a thin cubitus, hind-wing measuring 0·74×0·28 mm. Hinder legs longer than others, tibia 0·32 mm. long, tarsus—proximal joint 0·112 mm., distal joint 0·072 mm., tibia of front leg 0·21 mm., tarsus—proximal joint 0·080 mm., distal joint 0·064 ; paranychium acute. Mycetoma orange-yellow. Vasiform orifice with a rectangular operculum and lingula exposed, much elongated and armed with hairs on the tip.

(*Male*).—Length from vertex to the tip of claspers 1·06 mm. Concolourous with female. Antennae same as in the female, slightly shorter (for measurements see table below) wings corresponding to those of female, fore-wing measuring on an average 0·84×0·28 mm., hind-wing 0·70×0·23 mm. Abdomen tapering posteriorly ; claspers narrowing distally with tips curved inwards and beset with minute bristles. Penis slightly curved, smaller than claspers, measuring on an average 0·080 mm.

Measurements of antennae.

Segments	I	II	III	IV	V	VI	VII	Total length
Male	0·024	0·040	0·104	0·024	0·032	0·024	0·040	0·288 mm.
Female	0·024	0·048	0·104	0·024	0·032	0·040	0·048	0·320 mm.

Life-history.

Oviposition.—In nature, the eggs are laid singly, almost invariably, on the underside of the leaves, mostly on the top and middle leaves of a plant. In captivity, however, the eggs may be laid fairly frequently on the upper side of the leaves as well. The highest number of eggs laid by a single female in captivity has been 119, laid in 18 days. The average number of eggs laid by a female, in captivity, was 28 in 1929, and 43 in 1930. A maximum of 16 eggs may be laid in twenty-four hours, and the average number of eggs laid by a female in twenty-four hours was 6 in 1929, and 8 in 1930. The eggs are laid throughout the twenty-four hours. The oviposition period varies from 2 to 18 days. In laboratory experiments most of the females died earlier than their normal life because they got stuck in the moisture on the walls of the glass chimney of the breeding cage.

Table I.

Oviposition record, 1929-30.

Date	Particulars	Maximum number of eggs laid in 24 hours	Total number of eggs laid	Oviposition period (days)	Remarks
10th May 1930 .	Freshly emerged female from a cage*	..	34	4	*It was not definitely ascertained whether the females were fertilized.
21st June 1929 .	Newly emerged pair . .	9	19	3	
Do. .	,, ,, ,, . .	10	24	3	
2nd June 1930 .	Newly emerged female from a breeding cage* . . .	16	40	3	
Do. .	Newly emerged female from a breeding cage . . .	15	26	2	
13th June 1930 .	Newly emerged female from a breeding cage . . .	6	16	3	
Do. .	Newly emerged pair . .	11	27	3	

TABLE I—*contd.*

Date	Particulars	Maximum number of eggs laid in 24 hours	Total number of eggs laid	Oviposition period (days)	Remarks
19th June 1930 .	Newly emerged pair . .	9	35	5	
11th July 1929 .	„ „ „ . .	6	25	6	
22nd July 1929 .	Virgin female † . . .	11	71	14	† Eggs were laid parthenogenitically.
26th July 1929 .	Newly emerged pair . .	8	14	2	
6th Aug. 1929 .	„ „ „ . .	11	30	3	
14th Aug. 1929 .	Virgin female † . . .	7	22	5	
6th Sept. 1929 .	Newly emerged pair . .	7	22	5	
5th Oct. 1930 .	„ „ „ . .	13	119	18	
Do. .	„ „ „ . .	..	50	7	

Observations were made, in 1929 and 1930, to determine the influence of temperature on oviposition. A large number of freshly emerged adults (200 in 1929 and 100 in 1930) were enclosed in a cage over a cotton seedling and allowed to oviposit for one hour, after which they were removed to a fresh seedling. (These adults were obtained by keeping the infested cotton leaves in an ordinary parasite breeding cage. The imagines came into the tubes, which were kept in position from 8 a.m. to 5 p.m., when the adults were taken and counted, and were liberated in cages at 6 p.m.). The total number of eggs laid in an hour was recorded and continuous observations were made for twenty-four hours. It was observed that oviposition stopped when the air temperature went below 73°F. (Table II), and maximum oviposition occurred at a temperature above 80°F. These observations are admittedly far too few for establishing any definite correlation between temperature and oviposition.

For a small insect living on the under surface of a leaf, humidity must be considered as normally favourable and abundant, particularly in a cage where there is no movement of air.

TABLE II.

Influence of temperature on oviposition (1929-30).

1929 (200 adults)					1930 (100 adults)				
Date	Time of observation	Temperature °F.	No. of eggs laid	No. of eggs laid per 100 adults	Date	Time of observation	Temperature °F.	No. of eggs laid per 100 adults	Remarks
	hrs.					hrs.			
1st Oct.	19	88·0	7	4	28th Sept.	19	92·0	133	
	20	88·0	53	27		20	87·0	20	
	21	86·0	86	43		21	84·0	18	
	22	83·0	14	7		22	82·0	13	
	23	82·0	47	24		23	81·0	17	
	24	80·0	34	17		24	78·0	7	
2nd Oct.	1	78·0	25	13	29th Sept.	1	76·0	5	
	2	76·5	5	3		2	74·5	2	
	3	75·5	6	3		3	72·5	0	
	4	74·0	3	2		4	72·0	0	
	5	72·5	0	0		5	72·0	0	
	6	71·0	0	0		6	70·0	0	
	7	71·5	0	0		7	70·0	0	
	8	72·5	0	0		8	72·0	0	
	9	85·0	40	20		9	80·0	14	
	10	90·0	382	191		10	88·0	29	
	11	95·5	27	14		11	92·0	11	
	12	97·5	27	14		12	94·0	30	
	13	100·5	115*	58		13	100·0	10	* Eggs laid in 3 hours.
	15	103·0				14	100·5	4	
	16	101·5	13	7		15	100·0	3	
	17	98·5	5	3		16	98·0	4	
	18	96·0	4	2		17	96·5	4	
						18	94·0	3	

Incubation period.—The incubation period varies from 3 to 33 days, temperature being the chief controlling factor. During the main cotton growing season, *i.e.*, from April to September, the egg stage lasts from 3 to 5 days, during October and November it is prolonged from 5 to 17 days and during February and March it is 7 to 16 days. The longest incubation period so far observed was during December-January when it occupied 33 days (Table III).

Duration of the nymphal stage.—The duration of the three nymphal stages varies from 9-14 days from April to the end of September, but from October onward, this period is considerably prolonged, ranging from 17-73 days. In one case during 1928-29, the duration of the nymphal stages, at Lyallpur, was observed to have extended to 81 days during November-January (Table IV).

It is interesting to note that in *B. gossypiperda* the pupal stage is very short and occupies only 2-8 days, while in many other Aleurodidae, *e.g.*, the citrus

aleurodidæ, it is this stage which is the longest. The cottons are annuals, citrus perennials.

Duration of the life-cycles.—A complete life-cycle may occupy from 14-107 days. During April to September it occupies from 14-21 days. The shortest life-cycles have been observed during August. From October onward, the life-cycle is much prolonged and in one case it extended to 97 days (1930-31) between November and February, and the longest life-cycle so far recorded, at Lyallpur, has been 107 days in 1928-29. It is significant that it is the egg and the nymphal stages which are most prolonged, the pupal stage as stated above remains short (Table IV).

TABLE III.

Incubation period.

1929					1930				
Date of oviposition	Date of hatching	Incubation period (days)	Average temperatures of the period °F.		Date of oviposition	Date of hatching	Incubation period (days)	Average temperatures of the period °F.	
			Max.	Min.				Max.	Min.
22nd Feb.	9th Mar.	15	81·0	49·7					
27th Feb.	15th Mar.	16	83·7	50·6					
2nd Mar.	15th Mar.	13	84·5	50·5	2nd Mar.	13th Mar.	11	83·3	54·8
					16th Mar.	25th Mar.	9	87·0	53·7
					24th Mar.	31st Mar.	7	97·4	62·1
3rd Apl.	11th Apl.	8	91·3	63·6	* 8th Apl.	14th Apl.	6	..	..
23rd Apl.	27th Apl.	4	93·2	70·0	* 14th Apl.	19th Apl.	5	..	..
1st May	5th May	4	107·6	71·8	2nd May	6th May	4	95·3	82·6
					10th May	14th May	4	100·4	88·6
					28th May	1st June	4	100·5	89·2
					3rd June	8th June	5	100·4	87·7
					10th June	15th June	5	102·3	91·8
24th June	28th June	4	102·4	84·0	27th June	30th June and 1st July	3, 4	103·3	94·0
					8th July	12th July	4	103·8	87·3
14th July	18th July	4	102·5	81·1	15th July	19th July	4	97·8	84·7
					31st July	3rd and 4th Aug.	3, 4	99·6	82·2
7th Aug.	10th Aug.	3	94·3	84·9	6th Aug.	10th Aug.	4	99·2	82·4
					30th Aug.	4th Sept.	5	99·7	82·2
7th Sept.	11th Sept.	4	96·1	83·6	6th Sept.	11th Sept.	5	101·2	81·6
					10th Sept.	15th Sept.	5	99·4	82·6
1st Oct.	6th Oct.	5	88·5	67·1	3rd Oct.	10th Oct.	7	94·0	69·5
					24th Oct.	2nd Nov.	9	82·0	63·0
7th Oct.	13th Oct.	6	87·0	65·2	29th Oct.	14th and 15th Nov.	16, 17	73·2	62·3
2nd Nov.	12th Nov.	10	77·5	58·2	1st Nov.	16th and 18th Nov.	15, 17	78·1	61·1
6th Nov.	19th Nov.	13	76·9	56·2					
14th Nov.	30th Nov.	16	75·5	53·3	30th Nov.	2nd Jan. 1931.	33	69·7	47·4

* Temperatures not recorded.

TABLE

A few representative life-cycles of B.

Date of oviposition	Date of hatching	Incubation period (days)	Date of pupation	Nymphal period (days)	Date of emergence of imagines	Pupal period (days)	Duration of the total life-cycle (days)	Average temperatures during the period (°F.)	
								Max.	Min.
1929									
22nd Feb.	9th Mar.	15	..	..	26th Mar.	..	32	85·1	54·7
27th Feb.	15th Mar.	16	26th Mar.	11	29th ,,	3	30	86·6	55·4
2nd Mar.	15th Mar.	13	..	..	1st Apl.	..	30	87·7	56·4
3rd Apl.	11th Apl.	8	22nd Apl.	11	24th ,,	2	21	94·7	67·7
23rd Apl.	27th Apl.	4	..	..	9th May	..	16	101·3	71·3
1st May	5th May	4	16th May	11	18th, 19th May.	2, 3	17, 18	104·2	74·2
†24th June	28th June	4	12th July	14	15th July	3	21	..	..
14th July	18th July	4	28th ,,	10	30th, 31st July	2, 3	16, 17	95·2	81·9
23rd July	27th July	4	5th Aug.	9	7th, 8th Aug.	2, 3	15, 16	95·4	81·3
7th Aug.	10th Aug.	3	20th ,,	10	22nd Aug.	2	15	98·0	84·9
7th Sep.	11th Nov.	4	22nd Sep.	11	25th, 26th Sept.	3, 4	18, 19	96·2	82·7
1st Oct.	6th Oct.	5	20th Oct.	14	23rd, 26th Oct.	3	22, 25	90·3	67·2
7th Oct.	13th Oct.	6	30th ,,	17	5th, 11th Oct.	5	29, 35	85·9	62·2
2nd Nov.	12th Nov.	10	8th Jan. 1930.	57	13th, 15th Jan. 1930	5	73, 74	67·3	45·6
6th Nov.	19th ,,	13	..	..	14th, 15th Jan. 1930	..	69, 70	66·4	44·8
14th Nov.	30th ,,	16	..	..	2nd, 5th Feb. 1930	..	79, 82	63·8	42·6
1928*									
7th Nov.	20th Nov.	13	9th Feb. 1929	81	16th Feb. 1929	7	101	68·3	43·2
7th Nov.	22nd ,,	15	..	..	22nd Feb. 1929	..	107	68·6	43·3

*Only the longest life-cycles of 1928-29

†Temperatures

IV.

gossypiperda *in 1928, 1929 and 1930.*

Date of oviposition	Date of hatching	Incubation period (days)	Date of pupation	Nymphal period (days)	Date of emergence of imagines	Pupal period (days)	Duration of the total life-cycle (days)	Average temperatures during the period (°F.)	
								Max.	Min.
1930									
2nd Mar.	13th Mar.	11	29th Mar.	16	1st Apl.	3	30	88·5	57·4
16th ,,	25th ,,	9	5th Apl.	11	7th ,,	2	22	90·2	60·1
†24th ,,	31st ,,	7	11th, 12th Apl.	11, 12	13th, 14th Apl.	2	20, 21	..	..
†8th Apl.	14th Apl.	6	24th Apl.	10	26th Apl.	2	18	..	..
14th ,,	19th ,,	5	29th ,,	10	1st May	2	17	89·8	82·1
2nd May	6th May	4	16th May	10	18th, 19th May	2, 3	16, 17	97·6	85·8
10th ,,	14th ,,	4	23rd, 24th May	9, 10	25th, 26th May	2	15, 16	97·6	86·1
28th ,,	1st June	4	11th June	10	14th June	3	17	101·4	89·7
3rd June	8th ,,	5	17th ,,	9	19th ,,	2	16	101·1	89·5
10th ,,	15th ,,	5	25th ,,	10	27th ,,	2	17	101·1	85·1
27th ,,	30th ,, , 1st July	3, 4	10th July	9, 10	12th, 13th July	2, 3	15, 16	101·3	89·7
8th July	12th ,, ,	4	22nd ,,	10	23rd, 24th July	2	15, 16	100·2	85·1
15th ,,	19th ,,	4	29th ,,	10	31st July, 1st Aug.	2, 3	16, 17	99·0	84·8
31st ,,	3rd, 4th Aug.	3, 4	3rd, 4th, 12th Aug.	8, 9	14th, 15th Aug.	2, 3	14, 15	99·6	82·3
6th Aug.	10th Aug.	4	19th ,,	9	21st Aug.	2	15	100·1	82·7
30th ,,	4th Sept.	5	..	..	11th Sept.	..	18	99·3	80·3
6th Sept.	11th ,,	5	24th Sep.	13	27th ,,	3	21	94·8	80·2
10th ,,	15th ,,	5	28th ,,	13	1st Oct.	3	21	92·3	79·9
3rd Oct.	10th Oct.	7	24th Oct.	14	28th ,,	4	25	85·7	67·7
24th ,,	2nd Nov.	9	..	..	11th, 13th Dec.	..	48, 50	75·5	56·3
29th ,,	14th, 15th Nov.	16, 17	..	..	16th Dec. 21st Jan. 1931	..	49, 84	70·0	51·4
1st Nov.	16th, 18th Nov.	16, 17	..	..	21st Jan. 1931 5th Feb. 1931	..	82, 97	70·4	50·2
30th ,,	2nd Jan. 1931	33	15th Feb. 1931	44	20th Feb. 1931	5	82	69·9	48·8

are given for reference,
not recorded.

Emergence of the adults.—Emergence occurs, as a rule, during the day time. Observations made towards the end of September both in 1929 and 1930, showed that the emergence ceased altogether at night—between 7 p.m. and 7 a.m.—whereas, it was at its maximum between 8 and 11 a.m.

Longevity of the adults.—During summer the adults do not live very long and in captivity 2 to 5 days may be considered as their average life. During November, however, some adults lived up to 24 days in cages.

Parthenogenesis is a common phenomenon in the Aleurodidae and has been frequently observed in *B. gossypiperda*. Our observations, that only males develop from parthenogenetic eggs, agree with those of Morril and Back [1911] who studied this phenomenon in *Aleurodes citri*. Hargreaves [1915], however, obtained only females from the parthenogenetic eggs of *Aleyrodes vaporariorum* Westd.

Seasonal history.

The adults of the first brood of the year commence emerging from about the middle of January. Infestation, as a rule, starts on such weeds as *Convulvulus arvensis* and *Euphorbia* spp., and such cultivated plants as *Brassica rapa* and *Brassica oleracea*. From these the pest spreads to *Hibiscus esculentus*, the cucurbits and the ratoon cotton. No sooner the cotton crop has germinated in April than the White-fly appears on it and reproduces freely throughout the summer months. The maximum infestation on cotton occurs during July and August, more particularly in the latter month and then drops suddenly in September and October and henceforth continues much abated. About the end of the cotton season, the pest migrates to *Brassica* spp., and to various weeds and cultivated crops† where the immature stages of this insect pass their winter. In all, there may be twelve generations of this pest in the course of a year, but, as the broods overlap, practically all the stages are met with throughout the year.

Food plants.

B. gossypiperda is polyphagous and the following is the list of the food plants from which this pest has so far been recorded in the Punjab, both in the nymphal and pupal stages :—

Serial No.	English name	Botanical name	Family
1	Cotton . . .	*Gossypium* sp.*	Malvaceæ.
2	Hollyhock . . .	*Althea rosea*	,,
3	....	*Corchorus tridens*	,,
4	Lady's finger . .	*Hibiscus esculentus** . . .	,,
5	....	*Sida cordifolia*	,,

† List given under food plants.

Serial No.	English name	Botanical name	Family
6	Cabbage	*Brassica oleracea**	Cruciferæ.
7	Cauliflower	*Brassica oleracea* * var. *botrytis*	,,
8	Turnips	*Brassica rapa* *	,,
9	Rape-seed (Indian colza)	*Brassica campestris* * var. *sarson*	,,
10	Indian rape	*Brassica napus* var. *dichotoma*	,,
11	Radish	*Raphanus sativus*	,,
12	Melon	*Cucumis melo**	Cucurbitaceæ.
13	Water melon	*Citrullus vulgaris*	,,
14	Cucumber	*Cucumis sativus**	,,
15	Gourd	*Lagenaria vulgaris*	,,
16	....	*Cucumis pubescens***	,,
17	....	*Citrullus vulgaris* var. *fastulosus*	,,
18	....	*Momordica charantia*	,,
19	....	*Crotalaria juncea*	Leguminosæ.
20	Guara	*Cyamopsis psoraloides**	,,
21	....	*Melilotus parviflora**	,,
22	....	*Euphorbia pilulifera***	Euphorbiaceæ.
23	Red weed	*Euphorbia prostata*	,,
24	....	*Phylanthus* sp.	,,
25	Datura	*Datura alba***	Solonaceæ.
26	Chillies	*Capsicum frutescens*	,,
27	Tobacco	*Nicotiana tabacum**	,,
28	Black night-shade	*Solanum nigrum*	,,
29	Brinjal	*Solanum melongena**	,,
30	Potato	*Solanum tuberosum*	,,
31	....	*Solanum xanthocarpum*	,,
32	....	*Eclipta erecta*	Compositæ.
33	Sow thistle	*Sonchus oleraceus***	,,
34	Corn sow thistle	*Sonchus arvensis***	,,
35	Safflower	*Carthamus tinctorius*	,,
36	....	*Carthamus oxyacantha*	,,
37	....	*Boerhaavia diffusa*	Nyctaginaceæ.
38	....	*Celosia* sp.	Amarantaceæ.
39	....	*Achyranthes aspera*	,,
40	....	*Digera arvensis*	,,
41	White goose-foot	*Chenopodium album*	Chenopodiaceæ.
42	Bind weed	*Convulvulus arvensis***	Convulvulaceæ.
43	....	*Ipomaea* sp.	,,
44	Hul hul	*Cleome viscosa*	Capparidcæ.

NOTE.—The species attacking *Dalbergia sisso* appears to be different, and Roger Thomas' record [1932] requires confirmation.

* Cultivated food plants with comparativetly severe attack of White-fly.

** Weeds with severe attack.

CROSS INOCULATION.

To confirm the above observations and to determine definitely if *B. gossypiperda* could be bred on different host plants, the following cross inoculations were attempted. Adults were taken from different food plants and sleeved on to their other hosts. In every case the adults oviposited quite freely, the eggs hatched and the nymphs fed and reached maturity in a normal manner. Table V gives details of the observations so far made. Further work along this line is in progress.

TABLE V.

Cross inoculations, 1929-30.

Plant from which taken	Plant on which bred	Date of egg-laying	Date of hatching	Date of pupation	Date of emergence	Duration of life-cycle in days	Remarks
Gossypium sp.	*Brassica rapa*	2nd October 1929	....	....	25th, 26th October 1929.	23,24	
,,	*Brassica oleracea* var. *botrytis*	26th October 1929	8th November 1929	7th December 1929	15th December 1929	50	
,,	*Brassica rapa*	3rd, 6th November 1929.	14th, 15th November 1929.	....	18th, 22nd December 1929.	46,50	
,,	*Brassica oleracea*	19th April 1930	....	4th, 5th May 1930	5th, 7th May 1930	17,19	
Cucurbit	*Gossypium sp.*	19th July 1929	23rd July 1929	1st August 1929	4th, 5th August 1929	16,17	At Khanewal.
,,	,,	13th June 1930	17th, 18th June 1930.	27th June 1930	29th, 30th June 1930	16,17	
,,	,,	17th June 1930	21st, 22nd June 1930.	....	3rd, 4th July 1930	16,17	
,,	,,	3rd June 1930	7th, 8th June 1930	18th June 1930	20th June 1930	17	
Momordica charantia	,,	8th July 1930	12th July 1930	....	24th, 25th July 1930	16,17	
Althea rosea	,,	3rd April 1929	10th April 1929	....	23rd April 1929	20	
,,	,,	3rd April 1929	11th April 1929	22nd April 1929	24th April 1929	21	At Lyallpur.
Hibiscus esculentus	,,	6th August 1929	10th August 1929	19th August 1929	21st August 1929	15	
,,	,,	19th May 1930	23rd May 1930	....	3rd, 4th June 1930	15,16	
,,	,,	12th June 1930	16th June 1930	25th June 1930	27th, 29th June 1930	15,17	
,,	,,	8th July 1930	12th July 1930	23rd July 1930	25th July 1930	17	
Nicotiana tabacum	,,	5th May 1930	9th May 1930	19th May 1930	20th, 21st May 1930.	15,16	
,,	,,	9th May 1930	13th May 1930	22nd May 1930	24th, 28th May 1930.	15,19	At Khanewal.
Datura alba	,,	24th March 1930	31st March 1930	11th, 12th April 1930	13th, 14th April 1930	19,20	
Solanum malongena	,,	17th May 1930	21st May 1930	31st May 1930	2nd June 1930	16	
,,	,,	8th July 1930	12th July 1930	24th July 1930	26th July 1930	18	
Capsicum frutescens	,,	,,	,,	22nd July 1930	24th, 25th July 1930	16,17	
Brassica oleracea var. *botrytis.*	,,	6th November 1929	19th November 1929	....	14th, 15th January 1930.	69,70	

Relative incidence of White-fly infestation on different varieties of cotton grown under different conditions.

An accurate method of insect census is an essential foundation for a proper determination of the comparative infestation of a pest on different varieties of its hosts, and on plants grown under different conditions with reference to the date of sowing, application of manures, number of irrigations and variations in other agricultural operations. It must be admitted that at present no satisfactory method of taking an accurate census of *B. gossypiperda* is available. To obtain, as far as possible, average figures, three leaves were removed from each plant from three different regions—top, middle and bottom. From each plot under observation 9, or in some cases 18, leaves were taken from three or six different plants respectively. On each of these leaves all the immature stages present within a circle an inch in diameter were counted under a magnifying glass. Every effort was made to select these circles from different parts of the leaves at random. Tables VI-XIII show the results of these observations. It will be found that there are very wide fluctuations from leaf to leaf. Of course such fluctuations are in the very nature of things. On the same plant leaves from different regions may show an enormous difference as regards the population of this insect. Leaves from the same region from different plants may show equally large differences. In fact, leaves from the same region of the same plants may show wide variations in the number of the immature stages of the White-fly. Finally, different parts of the same leaf may show variations in the population of white-flies. Statistically, therefore, these results would be considered unsatisfactory. The relative value of these figures, however, is clear. For evident reasons it was not possible to examine a larger number of samples than has been done, or to carry out a more thorough examination.

Comparative infestation on different varieties of cotton.

It is commonly stated that the incidence of White-fly attack is higher on the broad-leaved varieties (Punjab-Americans) than on the narrow-leaved varieties (*desis*). Such is the opinion also of Misra and Lamba [1929], and according to Roberts [1929], of the broad-leaved, the attack is always worse on the 4F Punjab-American cotton and is much less on 289F Punjab-American. As stated above, to test these statements statistically is a very difficult matter. The following observations were made by the above-described method of estimating the population to verify the statements of different investigators.

The common varieties, 289F and 4F, representing the Punjab-Americans and *mollisoni* and *sanguineums* representing the *desis* were selected and sown side by side in the same plot and given identical agricultural treatments. Detailed observations made during the two seasons, 1929 and 1930, are given in the following tables.

TABLE VI.

Comparative infestation of White-fly on four varieties of cottons at Khanewal, (1929).

Note.—For each observation 9 leaves (3 top, 3 middle, 3 bottom) of each variety were examined. On each leaf a circle one inch in diameter was marked and all the immature stages present within the circle were counted.

Month	Dates of examination	Mollisoni						Sanguineum						289 F						4 F					
		Top three leaves		Middle three leaves		Bottom three leaves		Top three leaves		Middle three leaves		Bottom three leaves		Top three leaves		Middle three leaves		Bottom three leaves		Top three leaves		Middle three leaves		Bottom three leaves	
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
1929 JULY	6	91	29	57	61	..	3	11	13	24	14	..	..	2	..	5	2	..	..	4	1	7	11	9	9
	10	260	20	34	35	..	4	397	..	171	57	1	1	9	..	5	2	1	3	35	..	10	14	..	3
	13	87	86	16	45	3	13	144	55	18	36	1	11	7	1	5	6	2	5	40	20	14	12	15	9
	18	44	32	29	239	8	66	553	133	23	154	..	244	65	35	6	20	1	14	249	15	102	249	15	66
	24	579	433	139	84	5	55	79	..	11	2	3	5	24	..	3	..	4	2	944	7	96	185	7	27
	29	257	103	98	70	7	30	139	23	33	45	..	18	381	121	101	60	3	28	232	91	109	79	15	40
Total immature stages in 54 circles of one inch diameter.	..	3122						2429						923						2741					
Average number of immature stages in a circle.	..	58						45						17						51					
AUGUST	5	97	16	29	271	3	319	97	1	26	26	29	19	41	..	12	19	6	9	392	10	31	107	3	4
	13	557	251	71	175	3	14	108	30	41	34	47	26	1,211	81	671	507	24	31	497	359	133	217	7	150
	19	598	197	87	230	17	43	512	59	89	184	27	52	819	201	329	561	29	96	611	219	162	229	11	81
	25	473	276	113	266	31	70	314	114	99	337	9	75	721	417	223	489	116	241	419	302	103	344	23	100
Total immtaure stages in 36 circles.	..	4207						2355						6854						4514					

Average number of immature stages in a circle.	..	117						65						190						125			
SEPTEMBER	4	117	83	92	137	23	67	91	18	29	33	8	23	679	422	322	568	28	158	381	316	121	296	21	115
	10	71	53	33	85	19	34	31	17	17	19	5	16	234	141	87	281	17	102	190	149	63	133	13	100
	17	12	13	15	68	..	7	13	14	6	7	..	2	59	40	16	115	5	15	70	59	10	69	..	7
	23	26	4	6	..	..	..	12	1	18	4	..	..	66	23	6	22	..	32	392	..	8	75	..	32
	27	184	36	28	13	..	..	73	9	22	55	..	6	1261	85	21	61	..	2	948	38	33	160	..	..
Total immature stages in 45 circles.	..	1226						549						4818						3799					
Average number of immature stages in a circle.	..	27						12						107						84					
OCTOBER	4	7	2	1	8	..	5	33	14	16	14	..	2	46	10	..	4	1	9	132	27	2	45	..	4
	9	277	16	6	9	..	..	257	2	4	6	..	3	756	100	77	88	..	15	181	68	9	15	..	2
	16	188	11	11	7	2	..	193	73	5	11	..	8	693	96	142	86	5	15	497	6	248	74	..	4
	22	45	..	9	7	..	1	208	..	3	7	..	..	233	..	70	11	..	..	231	14	41	28	..	1
	27	63	..	21	5	..	2	187	..	4	11	..	..	202	..	33	27	..	..	263	..	33	81	..	..
Total immature stages in 45 circles.	..	703						1061						2719						2006					
Average number of immature stages in a circle.	..	16						24						60						45					
NOVEMBER	6	106	2	3	4	..	..	185	3	10	15	..	3	461	2	14	45	..	16	261	16	9	14	..	1
	10	132	1	6	1	..	10	75	34	4	13	..	8	594	55	..	20	..	2	144	..	..	10	..	..
	24	47	9	3	11	..	2	20	11	1	18	..	13	129	31	6	5	..	..	83	21	2	13	1	10
Total immature stages in 27 circles.	..	337						413						1380						585					
Average number of immature stages in a circle.	..	12						15						51						27					
DECEMBER	10	10	8	4	5	..	..	4	15	1	12	..	..	23	10	11	25	..	..	18	6	7	20	..	..
	17	6	60	..	8	6	68	6	62	2	15	8	77	7	12	5	5	12	17	11	11	..	8	11	14
Total immature stages in 18 circles.	..	175						202						127						101					
Average number of immature stages in a circle.	..	10						11						7						6					

TABLE VII.

Comparative infestation of White-fly on four varieties of cottons at Khanewal, (1930).

NOTE :—In 1930, eighteen leaves of each variety (6 top, 6 middle, 6 bottom) were examined. Figures for 16th and 20th June and from 1st September to 5th December have been consolidated, while those from 26th of June to 25th of August are given in full to show variations.

Month	Dates of examination	Plot number	Mollisoni						Sanguineum						289F						4F					
			Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leaves		Bottom six leaves	
			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
1930	16	90-94	8	9	2	7	..	3	6	1	4	6	..	5	6	4	3	6	..	4	8	5	..	7	..	3
JUNE	20	,,	14	5	8	18	1	5	14	2	3	12	..	3	11	5	3	12	..	8	13	4	2	16	..	5
	26	90	20	..	10	22	..	4	10	..	2	14	..	2	18	..	5	23	..	12	18	1	3	17	..	6
		91	30	..	13	38	..	7	8	5	2	16	..	3	17	..	1	36	..	11	13	..	3	13	..	10
		92	17	..	3	19	..	4	8	..	3	10	..	3	12	..	4	14	..	6	9	..	1	18	..	10
		93	13	1	3	17	..	8	7	..	3	12	1	5	9	..	3	6	..	8	9	..	4	16	..	9
		94	21	..	6	18	..	6	10	..	4	12	..	5	18	..	8	20	..	3	12	..	3	12	..	10
	30	90	29	6	14	25	..	5	10	3	3	11	..	5	10	10	8	11	..	9	6	1	14	14	..	7
		91	17	9	11	27	..	12	7	1	..	12	..	3	15	..	..	20	..	3	9	1	3	10	..	3
		92	10	9	4	18	..	7	6	..	9	9	2	3	9	1	1	14	..	4	10	8	7	12	..	3
		93	19	..	1	25	..	10	8	..	1	12	..	6	12	3	6	10	..	2	9	1	10	..	..	4
		94	28	..	8	15	..	4	10	..	..	4	..	4	20	..	6	8	..	7	15	..	1	12	..	4
Total number of immature stages in 360 circles.			663						330						485						424					
Average number of immature stages in a circle.			2						1						1						1					

| JULY | | |

Month	Dates of examination	Plot number	Mollisoni						Sanguineum						289F						4F					
			Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leaves		Bottom six leaves		Top six leaves		Middle six leave.		Bottom six leaves	
			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
AUGUST	4	90	86	58	56	117	8	95	35	54	36	84	4	79	55	57	41	107	..	95	60	68	48	105	11	98
		91	95	40	64	184	28	110	48	52	84	108	8	74	72	74	72	172	8	114	84	38	64	158	2	118
		92	108	136	74	172	6	124	84	78	40	126	9	100	88	92	58	162	18	104	78	60	68	132	2	116
		93	100	82	52	180	6	122	50	94	65	142	9	142	80	77	62	152	21	123	62	66	52	154	6	135
		94	116	84	52	200	15	152	65	68	86	132	4	88	109	91	96	137	9	168	80	74	60	118	18	156
	11	90	67	68	71	101	7	75	37	51	35	80	7	69	73	70	62	114	5	82	59	59	49	93	11	81
		91	63	62	69	90	9	79	41	52	37	85	8	67	72	76	62	112	8	79	56	65	52	92	10	87
		92	59	71	67	99	12	79	39	54	29	62	8	58	62	74	72	97	9	80	55	54	49	97	5	74
		93	53	72	37	83	11	80	55	55	42	78	10	60	62	73	42	76	13	71	71	86	52	84	9	58
		94	100	100	53	180	15	144	51	84	47	114	9	88	91	115	55	178	11	135	81	85	58	143	8	116
	20	90	42	56	39	102	14	80	29	27	19	67	6	49	38	36	32	78	9	74	34	43	21	71	8	67
		91	40	53	31	84	12	79	18	17	21	53	8	58	41	36	28	64	11	66	36	39	28	55	6	90
		92	45	45	36	94	14	86	20	23	24	57	11	56	34	37	31	67	10	68	41	38	22	53	5	74
		93	52	49	39	110	16	98	19	17	30	47	4	67	38	44	32	77	8	86	37	36	24	60	7	78
		94	56	55	41	110	24	115	21	25	36	66	6	56	46	48	30	75	6	95	41	39	22	50	9	83
	25	90	46	55	33	99	13	81	19	25	17	58	..	37	41	43	34	67	5	56	45	30	18	60	2	55
		91	33	69	60	51	..	42	26	23	14	43	..	24	72	47	49	54	..	39	44	51	33	41	2	49
		92	43	41	39	93	5	70	23	33	26	49	2	51	104	18	25	31	1	27	52	44	30	40	6	40
		93	55	40	51	90	9	80	14	20	31	53	2	71	57	37	48	86	5	67	36	40	27	59	..	90
Total number of immature stages in 360 circles.			7718						5178						7003						6301					
Average number of immature stages in a circle.			21						14						19						18					

SEPTEMBER	1	90-94	37	22	32	33	1	52	21	5	13	15	..	29	44	17	25	24	1	35	25	13	24	23	3	31
	10	,,	17	2	16	8	2	4	20	2	17	18	..	14	42	3	26	19	..	15	34	3	22	19	1	13
	16	,,	19	4	..	14	..	3	12	6	2	11	..	2	37	7	25	13	2	8	33	2	6	13	..	7
	22	,,	8	1	1	4	..	1	15	3	10	5	..	4	27	10	16	13	..	7	18	10	8	14	..	8
Total number of immature stages in 360 circles.			281						222						414						330					
Average number of immature stages in a circle.			0·7						0·6						1·1						0·8					
OCTOBER	1	90-94	8	1	2	7	..	4	18	5	9	8	..	6	40	4	13	18	..	11	25	3	6	14	..	10
	10	,,	7	..	..	2	..	5	16	4	8	11	..	6	22	6	14	13	..	10	23	2	13	23	..	12
	20	,,	4	..	1	10	..	..	14	3	8	10	..	4	21	5	9	16	..	8	15	1	6	13	..	7
	28	,,	2	..	5	7	..	2	10	2	7	10	..	11	17	2	6	14	..	8	11	2	7	9	..	9
Total number of immature stages in 360 circles.			67						170						257						212					
Average number of immature stages in a circle.			0·2						0·5						0·7						0·6					
NOVEMBER	8	90-94	4	1	3	5	..	..	9	3	4	13	..	9	11	1	5	14	..	12	11	2	8	9	..	9
	23	,,	2	..	1	3	..	2	9	5	4	13	..	10	8	2	2	12	..	7	5	3	4	11	..	4
Total number of immature stages in 180 circles.			21						79						74						66					
Average number of immature stages in a circle.			0·11						0·43						0·41						0·36					
DECEMBER	5	90-94	2	..	2	7	..	1	8	3	3	12	..	10	9	3	2	9	..	7	5	2	2	7	..	4
Total number of immature stages in 90 circles			12						36						30						20					
Average number of immature stages in a circle.			0·13						0·40						0·33						0·22					

Wide fluctuations in the numbers of the immature stages of the White-fly from leaf to leaf and plant to plant are quite evident. On the whole it appears that the average infestation on *mollisoni* was the highest early in the season, both during 1929 and 1930, later in the season (during August 1929 and during September, 1930) 289 F came in for an increased infestation (Fig. 1). During October and November of both years the severity of infestation was considerably reduced but 289 F was still leading, whereas *mollisoni* showed the least attack. *Sanguineums* which had shown the least attack earlier in the season, were the worst infested in December, although the general infestation was very light at the time. It may be noticed that *sanguineums* are very late varieties and when all the other varieties have ceased to grow, these continue to produce fresh leaves. Whatever be the statistical significance of the figures given, it is at any rate evident that the white-flies are no respectors of varieties of cotton and there appears to be no truth in the statement that the broad-leaved varieties are attacked more than the narrow-leaved and that 4 F carries a higher infestation than 289 F.* At this stage of our investigation we do not go further than this. Regarding the power of resistance, toleration or recovery of the different varieties no opinion can be expressed at present. In so far as the numbers of White-fly nymphs and pupae per unit area of the host plant are concerned the above statement holds.

Date of sowing and incidence of White-fly attack.

It has been stated by Roberts [1929] that the late sown cottons give higher yields, because they escape the attack of a few generations of the White-fly. With a view to test this assertion, experiments were started to determine the correlation between the date of sowing and the incidence of White-fly attack. The variety 289 F was sown at fortnightly intervals beginning from the 1st May to 1st July, 1930. Each sowing was replicated five times, each plot being 1/20 of an acre in area. The population of the pest was estimated by the method given above. The details of these observations are given in the following table (Table VIII).

* Roger Thomas [1932] maintains that 289 F is appreciably more resistant to White-fly than 4 F. He further states "All the local *desi* varieties are....reasonably though never totally resistant to White-fly attack". These assertions are not supported by actual facts.

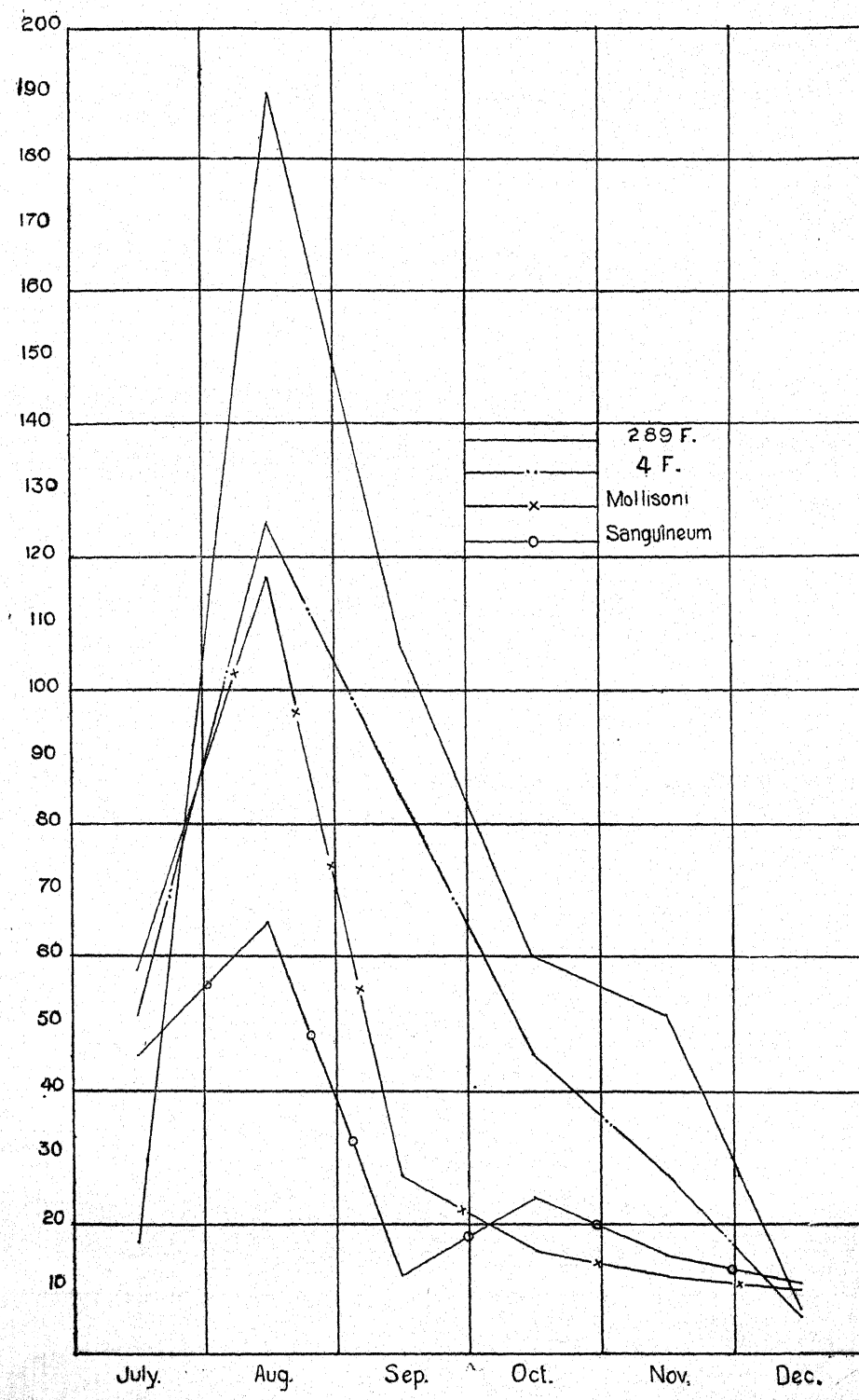

Fig. 1.—Comparative infestation of cotton White-fly on different varieties of cotton, 1929.

TABLE

Incidence of White-fly infestation on 289 F cotton at Khanewal in relation to the date of been consolidated. Figures from 12th July to

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Month	Date of examination	Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st May 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th May 1930					
				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
				Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
JUNE	14th June 1930 (5 observations)	42 47 52 57 62	45	10	..	2	19	..	3	..	30	12	..	..	14	..	6
Total immature stages in 90 circles.	....		..			34								32			
Average immature stages in a circle.	....		..			0·4								0·3			
	19th June 1930 (5 observations)	42 47 52 57 62	50	11	..	9	15	..	10	..	35	3	..	6	5	..	1
Total immature stages in 90 circles.	....		..			45								15			
Average immature stages in a circle.	....		..			0·5								0·2			
JULY	3rd July 1930 (5 observations)	42 47 52 57 62	64	97	12	21	95	..	23	..	49	39	4	3	43	..	6
Total immature stages in 90 circle.s	....	..	..			248								95			
Average i nmature stages in a circles.	....	..	..			3								1			
	5th July 1930 (5 observations)	42 47 52 57 62	66	108	16	16	137	..	68	..	51	49	4	2	56	..	18
Total immature stages in 90 circles.	....	..	..			345								129			
Average immature stages in a circle.	....	..	..			4								1·4			
	8th July 1930 (5 observations)	42 47 52 57 62	69	115	23	23	131	..	67	..	54	69	9	12	73	..	22
Total immature stages in 90 circles.	....	..	..			359								185			
Average immature stages in a circle.	....	..	..			4								2			

VIII

sowing. (*Figures from 14th June to 8th July and from 1st Septem ber to 8th December, have 25th August are given in full to show variations*).

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Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st July 1930					
		Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	32	20	..	1	14	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				36				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				0·4			..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	34	28	1	9	26	..	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				71				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				0·7				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	37	34	3	14	13	..	13	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				77				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
				0·8				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

TABLE 19

Month	Date of examination	Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st May 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th May 1930					
				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
				Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
JULY	12th July 1930	42	73	24	2	4	25	..	11	43	58	14	..	3	15	..	4
		47		20	1	6	17	..	8	48		8	..	5	11	..	2
		52		28	5	13	46	..	21	53		22	1	2	21	..	11
		57		26	6	8	38	..	13	58		18	2	5	20	..	6
		62		23	4	5	33	..	12	63		15	2	2	19	..	5
Total infestation in 90 circles.	....	..				399				..	..			213			
Average infestation in a circle.	....	..				4				..	..			2			
	18th July 1930	42	79	35	17	33	71	5	55	43	64	27	14	31	43	..	42
		47		34	20	29	64	7	49	48		23	8	25	50	3	35
		52		54	32	42	94	13	66	53		36	20	37	55	10	57
		57		44	21	39	85	9	65	58		32	14	34	53	7	56
		62		46	17	36	66	12	57	63		30	17	29	51	5	50
Total infestation in 90 circles.	....	..				1217				..				894			
Average infestation in a circle.	....	..				14				..				10			
	22nd July 1930	42	83	33	19	35	72	7	52	43	68	28	12	34	49	2	42
		47		34	21	32	62	5	54	48		25	5	28	44	5	34
		52		50	33	45	103	17	66	53		34	24	39	61	8	67
		57		46	25	37	92	15	63	58		32	14	36	54	4	50
		62		46	17	38	80	14	56	63		29	15	32	56	7	48
Total infestation in 90 circles.	....	..					1269			..				918			
Average infestation in a circle.	....	..					14			..				12			
	26th July 1930	42	87	38	23	39	88	8	55	43	72	31	38	37	57	3	49
		47		32	24	36	72	3	58	48		28	3	29	49	..	43
		52		75	44	61	137	12	92	53		40	26	45	81	7	76
		57		63	39	43	118	8	75	58		36	20	42	72	5	67
		62		61	29	45	104	10	66	63		33	21	40	62	9	62
Total infestation in 90 circles.	....	..				1558								1111			
Average infestation in a circle.	....	..				17								12			

VIII—*contd.*

30.

Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st July 1930					
		Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
44	41	1	..	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
49		2	..	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
54		3	..	3	1	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
59		2	..	2	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
64		1	..	3	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..			29				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
..	..			0·3				..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
44	47	14	6	19	12	9	21	45	33	2	..	3	2	1	2	..	..	..	..	..	..	..	..
49		8	5	12	11	7	13	50		..	..	2	3	..	4	..	..	..	..	..	..	..	..
54		22	9	29	25	9	38	55		4	2	6	2	3	4	..	..	..	..	..	..	..	..
59		19	9	25	24	11	38	60		3	2	4	4	2	4	..	..	..	..	..	..	..	..
64		18	9	21	21	8	31	65		2	1	3	4	2	3	..	..	..	..	..	..	..	..
..	..			503				..	..			74				..	..	..	..	..	..	..	..
..	..			6				..	..			1				..	..	..	..	..	..	..	..
44	51	17	8	22	13	12	19	45	37	3	..	2	2	1	4	1	..	..	..	..	..	..	..
49		9	3	17	14	7	14	50		1	..	3	1	2	1	2	..		..	..	..	..	..
54		25	8	32	31	7	44	55		6	2	8	5	5	5	3	..	..	..	..	..	..	..
59		18	10	27	24	13	37	60		4	3	5	3	3	3	4	..	..	..	..	..	..	..
64		20	15	23	17	5	29	65		2	..	4	3	..	2	5	..	..	..	..	..	..	..
..	..			540				..	..			88				..	..	..	..	..	..	..	..
..	..			6				..	..			1				..	..	..	..	..	..	..	..
44	55	19	11	24	20	5	25	45	41	3	..	5	3	2	4	1	26	2	..	1	1	2	1
49		16	5	20	13	3	20	50		3	..	7	3	4	5	2		2	..	2	3	1	4
54		36	6	38	48	8	54	55		10	4	15	10	2	10	3		4	..	3	3	2	3
59		23	12	28	31	8	45	60		8	3	8	14	3	5	4		2	..	1	1	1	..
64		31	8	32	41	10	49	65		9	..	11	6	1	8	5		1	..	2	1	1	1
..				690								166								45			
..				9								2								0·5			

TABLE 19

Month	Date of examination	Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st May 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th May 1930					
				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
				Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
AUGUST	2nd Aug. 1930	42	94	51	34	42	106	11	66	43	79	35	11	39	62	5	59
		47		44	29	39	92	8	59	48		33	12	31	56	2	51
		52		82	68	74	162	18	117	53		41	25	48	80	8	72
		57		71	45	49	136	13	97	58		39	23	44	74	7	65
		62		62	37	51	123	9	81	63		36	17	41	66	8	65
Total infestation in 90 circles.						1876								1155			
Average infestation in a circle.						20								13			
	8th Aug. 1930	42	100	52	38	49	114	10	69	43	85	42	18	41	65	7	66
		47		48	36	37	94	7	59	48		38	15	32	61	3	65
		52		91	77	78	132	20	126	53		51	26	44	74	12	73
		57		88	49	56	135	16	103	58		48	30	48	75	5	80
		62		65	53	54	129	12	85	63		55	32	51	87	14	84
Total infestation in 90 circles.						1982								1342			
Average infestation in a circle.						22								15			
	19th Aug. 1930	42	111	39	29	27	59	8	45	43	96	25	11	18	23	..	20
		47		24	9	19	21	2	23	48		22	7	16	16	1	20
		52		31	8	21	31	..	22	53		34	17	29	34	5	45
		57		32	21	23	46	3	38	58		27	10	25	27	3	36
		62		28	14	25	46	2	33	63		30	9	23	26	..	29
Total infestation in 90 circles.						729								588			
Average infestation in a circle.						8								7			
	25th Aug. 1930	42	117	13	7	21	7	..	11	43	102	13	22	14	18	..	15
		47		19	22	11	18	2	9	48		21	15	12	11	..	17
		52		15	9	13	12	..	12	53		9	16	10	19	1	19
		57		12	19	6	19	..	14	58		10	16	15	20	..	21
		62		15	17	10	20	1	19	63		13	15	7	19	2	15
Total infestation in 90 circle.						353								385			
Average infestation in a circle.						4								4			

VIII—*contd.*

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Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st July 1930					
		Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
44	62	23	8	35	31	7	40	45	48	7	2	8	8	5	10	1	33	2	..	3	3	1	4
49		19	4	29	19	4	26	50		8	4	11	7	5	9	2		3	..	4	3	3	4
54		39	14	44	64	10	65	55		11	2	8	14	4	15	3		5	1	6	4	5	4
59		32	9	41	49	7	61	60		14	3	11	9	5	15	4		4	2	5	2	3	2
64		28	14	37	49	9	55	65		9	3	10	8	3	14	5		3	..	2	1	1	4
				872								243								84			
				10								3								1			
44	68	27	8	38	38	5	49	45	54	10	1	12	11	7	11	1	39	5	..	4	6	3	6
49		24	8	32	26	4	29	50		9	5	15	13	6	13	2		7	1	5	4	5	7
54		42	17	40	79	12	68	55		12	5	15	20	15	24	3		10	2	8	6	7	10
59		37	10	41	41	5	53	60		17	2	4	16	12	18	4		7	2	6	5	5	5
64		38	14	44	45	8	61	65		14	5	13	13	13	20	5		5	..	3	2	3	5
				912								352								144			
				10								4								2			
44	79	21	8	22	18	..	26	45	65	12	..	11	9	..	13	1	48	2	..	3	1	..	4
49		18	15	16	13	..	17	50		8	..	9	2	..	9	2		3	..	2	4	1	4
54		32	17	27	24	4	44	55		15	..	13	17	3	25	3		5	..	4	8	..	8
59		25	10	24	24	1	33	60		11	..	13	12	..	14	4		3	..	2	4	3	6
64		28	14	25	30	2	38	65		13	1	12	17	2	18	5		4	..	1	4	..	3
				576								259								79			
				6								3								1			
44	85	12	17	10	18	1	17	45	71	15	7	7	14	..	3	1	56	25	5	10	12	2	22
49		18	13	9	13	..	12	50		15	10	9	14	2	1	2		20	12	4	18	2	22
54		22	6	4	14	1	18	55		10	12	6	16	..	18	3		10	8	8	18	..	20
59		12	11	11	13	..	9	60		11	13	12	29	4	23	4		17	7	5	19	1	27
64		13	12	8	12	..	10	65		20	14	8	12	1	27	5		15	7	2	14	1	20
				316								347								353			
				3								4								4			

D

TABLE

19

Month	Date of examination	Date of sowing 1st May 1930								Date of sowing 15th May 1930							
		Plot No.	Age of plant on the day of examination (days)	Top six leaves		Middle six leaves		Bottom six leaves		Plot No.	Age of plant on the day of examination (days)	Top six leaves		Middle six leaves		Bottom six leaves	
				Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
SEPTEMBER	1st Sep. 1930 (4 obervations)	,,	124	90	42	51	137	14	129	,,	109	78	44	43	104	5	124
Total infestation in 72 circles						463								398			
Average infestation in a circle						6								5			
	11th Sep. 1930 (5 observations)	,,	134	35	1	28	8	..	6	,,	119	36	3	23	11	1	11
Total infestation in 90 circles						78								85			
Average infestation in a circle						0·9								1			
	19th Sep. 1930 (3 observations)	,,	142	15	3	4	8	..	2	,,	127	11	1	3	5	..	1
Total infestation in 54 circles						32								21			
Average infestation in a circle						0·6								0·4			
OCTOBER	3rd Oct. 1930 (3 observations)	,,	156	18	3	11	7	..	7	,,	141	12	4	11	15	..	7
Total infestation in 54 circles						46								49			
Average infestation in a circle						0·8								0·9			
	16th Oct. 1930 (3 observations)	,,	169	15	1	9	7	..	2	,,	154	13	3	5	7	..	7
Total infestation in 54 circles						34								35			
Average infestation in a circle						0·6								0·6			
	30th Oct. 1930 (3 observations)	,,	183	10	2	6	6	1	6	,,	168	5	2	4	8	1	7
Total infestation in 54 circles						31								27			
Average infestation in a circle						0·6								0·5			
NOVEMBER	11th Nov. 1930 (3 observations)	,,	194	9	2	4	6	..	5	,,	179	7	2	4	9	..	6
Total infestation in 54 circles						26								28			
Average infestation in a circle						0·5								0·5			
	19th Nov. 1930 (3 observations)	,,	202	7	1	4	9	..	7	,,	187	6	2	3	9	1	7
Total infestation in 54 circles						28								28			
Average infestation in a circle						0·5								0·5			
DECEMBER	8th Dec. 1930 (3 observations)	,,	221	7	1	3	7	..	3	,,	206	4	4	2	4	..	6
Total infestation in 54 circles						21								20			
Average infestation in a circle						0·4								0·4			

VIII—*contd.*

30

Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 15th June 1930						Plot No.	Age of plant on the day of examination (days)	Date of sowing 1st July 1930					
		Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves				Top six leaves		Middle six leaves		Bottom six leaves	
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ			Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ
,,	92	71	39	39	103	..	103	,,	78	52	38	34	74	7	64	,,	61	50	27	25	51	6	35
				284								269								194			
				4								4								3			
,,	102	31	..	15	5	..	9	,,	88	21	3	8	14	1	11	,,	72	25	13	11	13	3	14
				60								58								79			
				0·7								0·6								0·9			
,,	110	8	1	3	14	4	..	,,	96	6	1	2	9	..	1	,,	80	6	1	1	2	..	6
				30								19								16			
				0·6								0·3								0·3			
,,	124	9	2	6	5	4	5	,,	110	14	..	7	4	..	1	,,	94	0	..	4	10	..	1
				31								26								24			
				0·6								0·5								0·4			
,,	137	12	2	5	9	2	6	,,	123	9	3	4	9	..	6	,,	107	7	5	5	5	..	4
				36								31								26			
				0·7				..				0·6								0·5			
,,	151	6	4	3	10	..	8	,,	137	7	1	5	4	2	6	,,	121	9	1	5	6	1	4
				31								25								25			
				0·6								0·5								0·5			
,,	163	7	1	4	9	1	5	,,	148	7	2	3	6	..	6	,,	132	7	1	3	10	..	6
				27								24								27			
				0·5								0·4								0·5			
,,	171	5	2	3	6	..	3	,,	156	6	..	5	6	..	7	,,	140	7	2	1	9	..	7
				19								24								26			
				0·3								0·4								0·5			
,,	190	6	3	3	9	..	5	,,	175	6	3	4	7	2	5	,,	159	5	4	2	8	..	7
				26						..		27								26			
				0·5						..		0·5								0·5			

Three different tests may be employed when comparing the incidence of attack on the plants sown on different dates, and, therefore, at different stages of growth at a particular time :—

(*i*) The incidence of attack may be determined on a particular date irrespective of the age of the plants to be compared.

(*ii*) The incidence of attack on the plants of the same age may be compared.

(*iii*) The total infestation on the plants during their entire life may be compared.

We shall examine the data collected in accordance with these tests.

(*i*) *Relative incidence of attack on specific dates on 289F cotton plants sown on different dates.*—The observations made so far (Table VIII) undoubtedly show that, up to the 19th August 1930, the earlier sown plants were more severey infested than the later sown.

A week later, however, *i.e.*, on the 25th August, the situation had altered and practically all the five lots showed almost the same intensity of attack, and henceforward, although the severity of attack had abated, yet its relative incidence was more or less the same on the early sown and late sown crops, as shown in Table IX in which the results of Table VIII have been summarised for convenience of comparison.

TABLE IX.

Incidence of attack on particular dates on plants sown at different dates.

(Summary of Table VIII.)

Date of examination	Date of sowing										Remarks
	1st May 1930		15th May 1930		1st June 1930		15th June 1930		1st July 1930		
	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymph and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	
1930.											
18th July	79	156	64	113	47	54	33	7	..	..	Average number of nymphs in 18 circles, each one inch in diameter.
26th „	87	205	72	145	55	100	41	15	26	4	
8th Aug.	100	260	85	168	68	107	54	36	39	12	
19th „	111	89	96	66	79	66	65	27	50	9	
25th „	117	43	102	52	85	39	71	45	56	46	

TABLE IX—*contd.*

Incidence of attack on particular dates on plants sown at different dates—contd.

(Summary of Table VIII).

Date of examination	Date of sowing										Remarks
	1st May 1930		15th May 1930		1st June 1930		15th June 1930		1st July 1930		
	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	
1930.											
1st Sept.	124	77	109	68	92	61	78	44	63	33	Average number of nymphs in 18 circles, each one inch in diameter.
11th „	134	3	119	5	102	3	88	6	73	8	
3rd Oct.	156	6	141	9	124	4	110	2	95	4	
30th „	183	5	168	6	151	7	137	4	122	4	
19th Nov.	203	6	188	6	171	4	157	4	142	6	
8th Dec.	222	4	207	5	190	6	176	5	161	6	

(*ii*) *Incidence of attack on the plants of the same age.*—On the other hand, if the plants of the same age, from different sowings be compared then one finds a different story (Fig. 2). For instance, in the plants about 50 days old, the earliest and the latest sown are the least attacked, while those sown on intermediate dates show higher attack, and the crop sown on 1st June is the worst infested. At the age of 64 days those sown on 15th May and 1st June show the highest attack, while on the other three the attack is more or less similar. The plants sown on 15th May have five times higher attack than those sown on 1st May. If plants of different sowings are compared at the age of 79-81 days, then one finds that the earlier sown are the more heavily infested, and, age for age, the difference becomes marked as we proceed towards the later sown. At the ages of 94-96 days the earliest sown is by far the worst attacked and the lots sown on 15th June and 1st July are the least attacked. At the age of 124 days the earliest sown is the only plot which is seriously infested. At the age of 137 to 142 days the attack may be stated to be the same in all cases, and this continues as shown below:

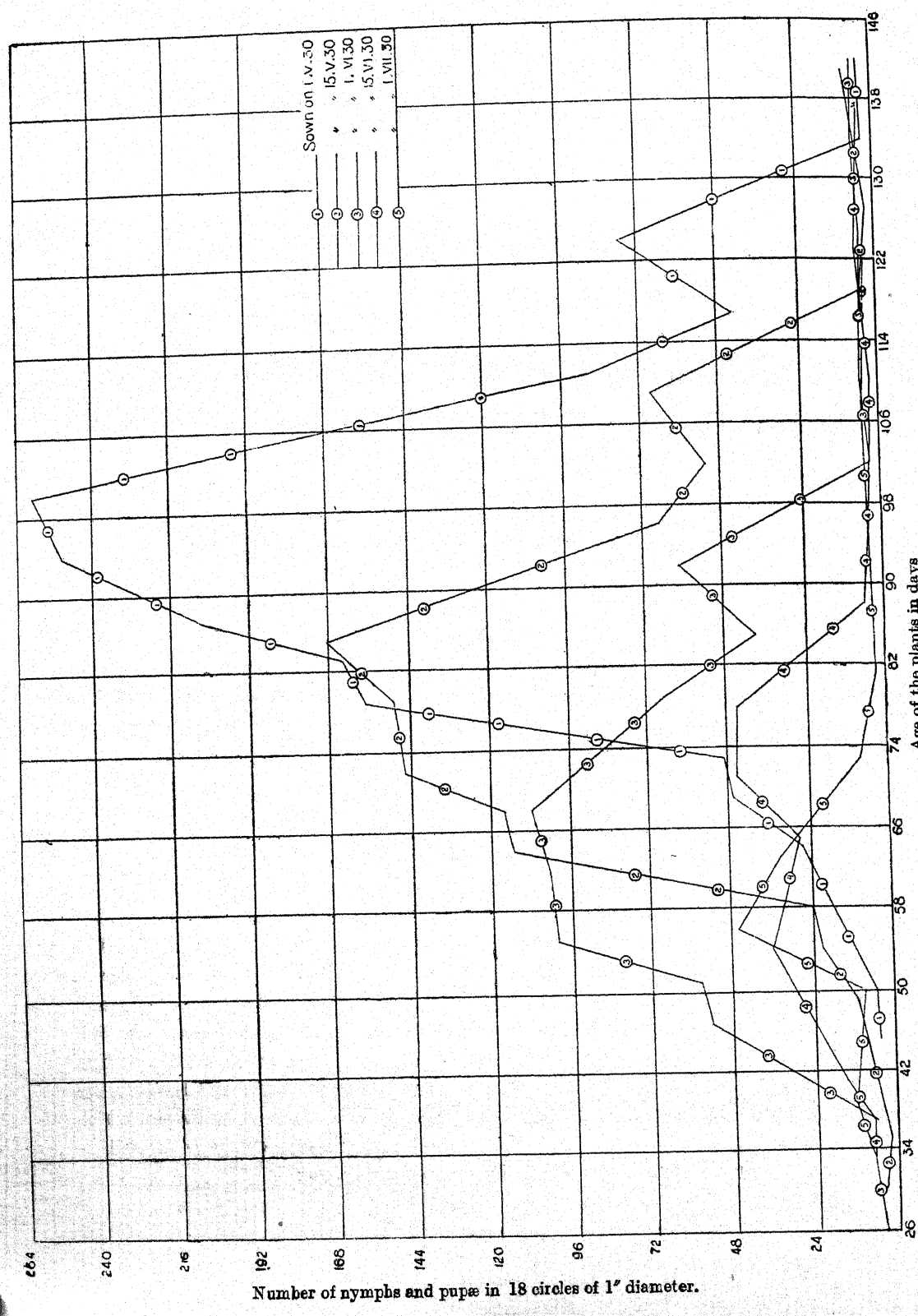


Fig. 2.—Infestation of *B. gossypiperda* on plants of same age but sown at different dates.

TABLE X.

Incidence of White-fly infestation on plants at approximately the same ages sown at different dates.

(Summary of Table VIII).

Date of sowing										Remarks
1st May 1930		15th May 1930		1st June 1930		15th June 1930		1st July 1930		
Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	
50	5	51	16	51	57	54	36	50	9	Average number of nymphs and pupæ in 18 circles of one inch diameter each.
64	26	64	113	62	102	65	27	63	33	
79	156	79	148	79	66	78	44	81	3	
94	250	96	66	92	61	96	9	95	4	
124	77	127	2	124	4	123	6	122	4	
142	4	141	9	137	6	137	4	142	6	

(*iii*) *Total infestation during the entire life of the plant.*—It will be quite obvious from Table XI and Fig. 3, that the plants sown on 15th June and 1st July had a very low total infestation. The plots sown on 1st of May, 15th of May and on 1st of June did not show any difference in the early stages and all three had suffered from a relatively bad attack. The total magnitude of attack is highest on the early sown and is gradually less on the later sown. Unfortunately, the plots available for these experiments were not of uniform fertility. Some of these plots had remained fallow before cotton, while on others cotton had followed wheat. The yields* obtained, therefore, cannot be compared. In a general way, it may be stated that the four plots sown from the 1st of May to the 15th of June gave more or less similar yield, while the one sown on 1st July gave an excessive yield, evidently because of high soil fertility. It will not be out of place to mention that Trought [1930] has shown that, the cottons sown late have a greater rate of growth and increased flower and boll production, and he is inclined to recommend 1st June to 15th June for general sowing. He, however, remarks : " It seems clear also that sowing as late as July 1st, is not profitable and first half of June is probably the

*Sown on	Yield per acre Mds.	Srs.
1st May 1930	9	16
15th „ „	10	6
1st June „	8	9
15th „ „	8	16
1st July „	17	27

optimum date." It is possible that the infestation of White-fly is a contributing factor and the late sown may possibly give a higher yield because of a less severe attack. However, one has to recognise that it is really the plants sown after the 15th June which are the least infested. The plants sown from 1st May to 1st June are in a peculiar situation. The earlier sown have the greater total infestation while the later sown get a more serious attack at the earlier stage of their life. It is too early to express a definite opinion.

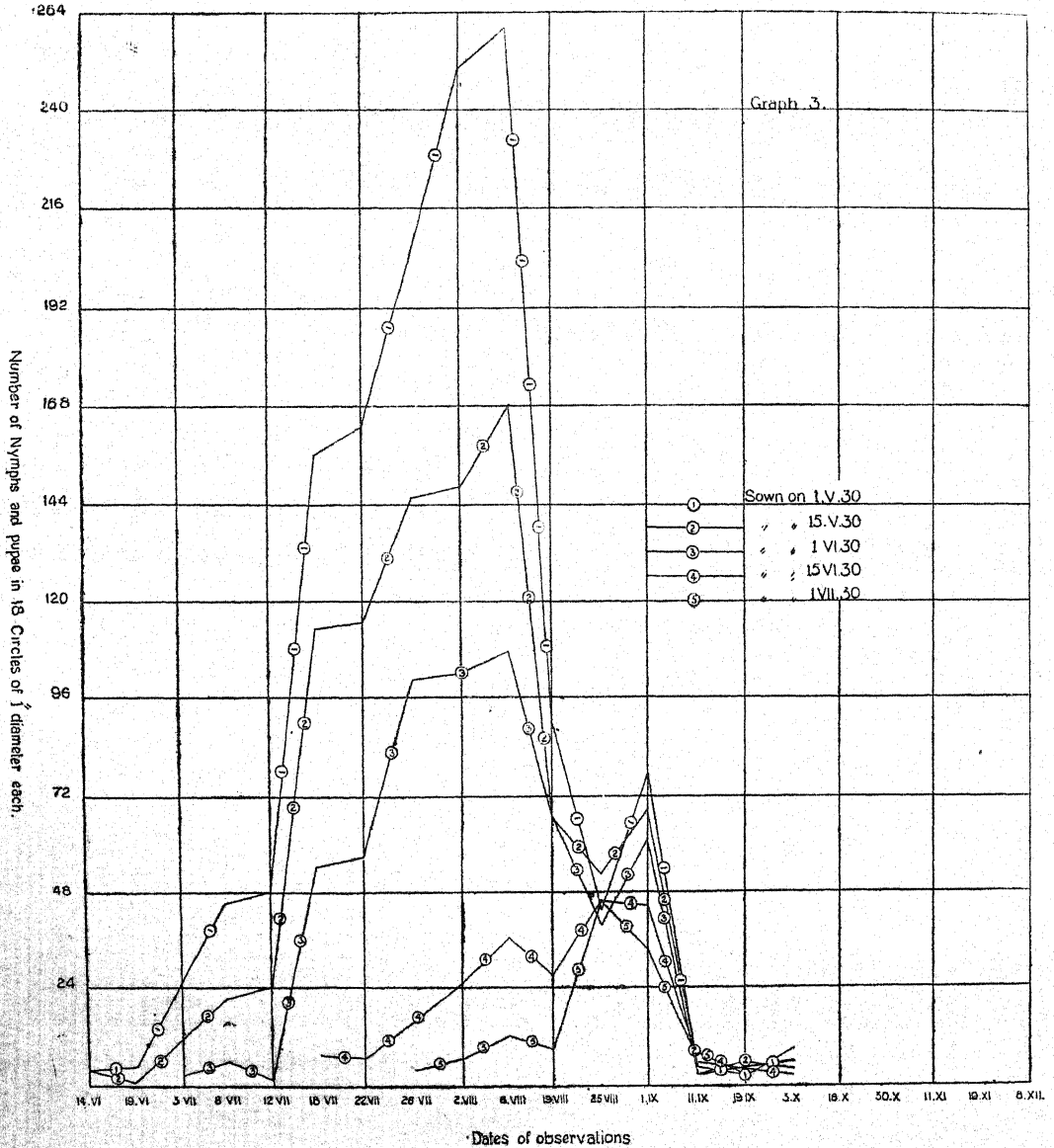


Fig. 3.—Infestation of *B. gossypiperda* on plants sown on different dates.

N.B.—From 3rd October 1930 to 8th December 1930, the infestation was practically uniform on all sowings hence it is not represented in the graph.

TABLE XI.

Relative incidence of White-fly infestation on plants sown at different dates.

(Summary of Table VIII.)

Date of examination	Date of sowing: 1st May 1930		15th May 1930		1st June 1930		15th June 1930		1st July 1930		Remarks
	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	Age of plants (days)	No. of nymphs and pupæ	
1930											
14th June	45	4	30	4							The number of nymphs in this table is an average number present on an area of 18 circle inches.
19th ,,	50	5	35	1							
3rd July	64	26	49	11	32	3					
8th ,,	69	46	54	21	37	6					
12th ,,	73	48	58	24	41	1					
18th ,,	79	156	64	113	47	54	33	7			
22nd ,,	83	163	68	115	51	57	37	7			
26th ,,	87	205	72	145	55	100	41	15	26	4	
2nd Aug.	94	250	79	148	62	102	48	75	33	7	
8th ,,	100	260	85	168	68	107	54	36	39	12	
19th ,,	111	89	96	66	79	66	65	27	50	9	
25th ,,	117	43	102	52	85	39	71	45	56	46	
1st Sept.	124	77	109	68	92	61	78	44	63	33	
11th ,,	134	3	119	5	102	3	88	6	73	8	
19th ,,	142	4	127	2	110	5	96	4	81	3	
3rd Oct.	156	6	141	9	124	4	110	2	95	4	
16th ,,	169	3	154	6	137	6	123	6	108	5	
30th ,,	183	5	168	6	151	7	137	4	122	4	
11th Nov.	195	4	180	6	163	5	149	5	134	6	
19th ,,	203	6	188	6	171	4	157	4	142	6	
8th Dec.	222	4	207	5	190	6	176	5	161	6	
Total		1,407		981		636		292		153	
Average per observation		69		47		33		18		11	

Influence of manures and fertilizers on the incidence of cotton White-fly.

The increase in the vigour of the plant through manures and the change in the chemical nature of the sap produced through fertilizers, are considered by some as measures likely to safeguard crops against damage from insect pests [Andrews, 1923]. During 1929, a few preliminary field experiments were made to study the effect of manures on the infestation of *B. grossypiperda*. Farmyard manure at the rate of one hundred maunds per acre was applied a couple of days before

sowing and soda nitrate and ammonium sulphate, each at one maund per acre, were applied on the 10th of June just before sowing. For each manure an area of half an acre was treated and a corresponding area was kept as control. Both the manured and unmanured areas were further divided into two equal plots and for each experiment one manured and one unmanured plot was put under 289F, and the other under *mollisoni*. The whole area was sown on the 10th of June and except the manurial differences, all other operations were precisely uniform in all the plots. Except during July and December, 289F was twice as severely infested as *mollisoni*, and the manured plots on the whole showed a slightly higher infestation than the unmanured. Of the manured plots the White-fly attack on the whole was severest in plots treated with farmyard manure, while it was lowest in the area treated with soda nitrate. The difference, however, cannot be considered significant.

A similar experiment was repeated in 1930, with soda nitrate and ammonium sulphate which were applied on 15th August 1930 as against 10th June 1929 in the previous experiment. The White-fly attack being very mild this year, the infestation was practically uniform in both manured and control plots. Soda nitrate, however, showed a slight indication of lower infestation than the others but the difference was insignificant.

TABLE XII.

Incidence of White-fly attack in relation to farmyard manure and fertilizers.

1929.

Month under observation	No. of observations	Farmyard manure		Control	Soda nitrate	Control	Ammonium sulphate	Control
		Immature stages		Immature stages	Immature stages	Immature stages	Immature stages	Immature stages
		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter	(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter	(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter	(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter	(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter
1929								
July	4	*mollisoni*	(1) 205	(1) 218	(1) 401	(1) 262	(1) 184	(1) 142
			(2) 23	(2) 24	(2) 45	(2) 29	(2) 20	(2) 16
		289-F	(1) 33	(1) 59	(1) 67	(1) 56	(1) 63	(1) 59
			(2) 4	(2) 7	(2) 7	(2) 6	(2) 7	(2) 7
August	2	*mollisoni*	(1) 693	(1) 212	(1) 1,002	(1) 257	(1) 947	(1) 259
			(2) 77	(2) 24	(2) 111	(2) 29	(2) 105	(2) 29
		289-F	(1) 1,468	(1) 1,044	(1) 1,573	(1) 908	(1) 1,654	(1) 1,046
			(2) 163	(2) 116	(2) 175	(2) 101	(2) 184	(2) 116

TABLE XII—*contd.*

Incidence of White-fly attack in relation to farmyard manure and fertilizers—contd.

1929—contd.

Month under observation	No. of observations	Farmyard manure		Control		Soda nitrate		Control		Ammonium sulphate		Control	
		Immature stages		Immature stages		Immature stages		Immature stages		Immature stages		Immature stages	
		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter		(1) Per observation of 9 leaves (2) In a circle of 1 in. diameter	
1929													
September	2	*mollisoni*	(1) 118	(1) 105		(1) 77		(1) 40		(1) 77		(1) 229	
			(2) 13	(2) 12		(2) 8		(2) 4		(2) 8		(2) 25	
		289-F	(1) 596	(1) 605		(1) 376		(1) 175		(1) 565		(1) 379	
			(2) 66	(2) 67		(2) 42		(2) 19		(2) 63		(2) 42	
October	4	*mollisoni*	(1) 271	(1) 72		(1) 74		(1) 124		(1) 59		(1) 143	
			(2) 30	(2) 8		(2) 8		(2) 14		(2) 7		(2) 16	
		289 F	(1) 316	(1) 298		(1) 209		(1) 583		(1) 285		(1) 330	
			(2) 35	(2) 33		(2) 23		(2) 65		(2) 32		(2) 37	
November	2	*mollisoni*	(1) 132	(1) 120		(1) 96		(1) 97		(1) 88		(1) 96	
			(2) 15	(2) 13		(2) 11		(2) 11		(2) 10		(2) 11	
		289-F	(1) 296	(1) 266		(1) 259		(1) 181		(1) 269		(1) 257	
			(2) 33	(2) 29		(2) 29		(2) 20		(2) 30		(2) 29	
December	2	*mollisoni*	(1) 55	(1) 57		(1) 35		(1) 85		(1) 26		(1) 64	
			(2) 6	(2) 6		(2) 4		(2) 9		(2) 3		(2) 7	
		289-F	(1) 7	(1) 3		(1) 9		(1) 6		(1) 22		(1) 8	
			(2) 1	(2) 0·3		(2) 1		(2) 1		(2) 2		(2) 1	

TABLE XIII.

Incidence of White-fly attack in relation to fertilizers (1930).

Date of examination	Plot number	Soda nitrate						Plot number	Ammonium sulphate						Plot number	Unmanured (control)						Remarks
		Top six leaves		Middle six leaves		Bottom six leaves			Top six leaves		Middle six leaves		Bottom six leaves			Top six leaves		Middle six leaves		Bottom six leaves		
		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ		Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	Eggs	Nymphs and pupæ	
1930—																						
22nd August	46	85	38	18	74	2	61	56	73	56	30	31	..	43	49	102	73	25	55	..	75	
	51	99	43	25	59	..	55	61	75	53	22	82	4	67	59	28	11	13	22	..	22	
27th ,,	46	26	30	19	48	6	45	56	27	23	70	57	18	46	49	108	49	18	55	12	52	
	51	86	40	45	43	9	62	61	28	16	26	36	13	52	59	25	23	17	40	3	47	
30th ,,	46	18	16	9	18	2	28	56	23	25	14	21	4	36	49	26	30	18	24	1	40	
	51	20	21	12	24	2	32	61	45	23	10	26	3	37	59	29	30	16	22	3	45	
Total immature stages in 18 circles				203							203							193				
Average immature stages in a circle				11·3							11·3							10·7				
12th September	46	6	4	4	2	..	7	56	10	3	3	3	1	4	49	12	5	4	5	..	5	
	51	8	2	3	4	1	4	61	7	5	5	4	..	5	59	10	5	4	3	1	3	
19th ,,	46	6	3	3	4	..	4	56	7	3	2	3	1	6	49	10	6	5	4	2	5	
	51	4	4	3	6	..	3	61	5	4	4	5	..	8	59	9	4	4	6	1	7	
Total immature stages in 18 circles				21							25							31				

Average immature stages in a circle				1·1							1·4						1·7				
3rd October	46,51	7	4	4	7	..	5	56,61	8	6	5	7	..	7	49,59	11	8	6	8	1	5
10th ,,	,,	7	7	5	3	1	5	,,	9	6	5	8	1	8	,,	13	7	7	8	1	8
18th ,,	,,	6	8	4	4	1	6	,,	7	4	6	5	2	6	,,	11	7	5	8	1	8
25th ,,	,,	5	3	2	3	..	1	,,	5	7	2	5	1	2	,,	9	11	5	5	1	5
31st ,,	,,	5	4	1	3	1	4	,,	7	5	3	2	..	2	,,	10	7	3	5	1	1
Total immature stages in 18 circles				12							14							19			
Average immature stages in a circle				0·7							0·8							1·0			
7th November	46,51	5	3	1	3	..	3	56,61	6	3	3	3	1	2	49,59	7	5	4	3	1	4
18th ,,	,,	9	5	3	6	..	4	,,	7	4	1	4	1	3	,,	7	3	4	4	2	3
Total immature stages in 18 circles				11							10							12			
Average immature stages in a circle				0·6							0·6							0·7			
3rd December	46,51	7	3	2	2	..	1	56,61	5	3	1	3	..	2	49,59	9	3	3	1	..	6
18th ,,	,,	4	1	1	4	..	6	,,	3	2	2	4	1	6	,,	5	2	1	5	..	6
Total immature stages in 18 circles				8							8							10			
Average immature stages in a circle				0·4							0·4							0·6			

Figures of two observations of each date have been consolidated from 3rd October 1930 onwards.

NATURE AND EXTENT OF DAMAGE.

To determine the nature and extent of damage caused by *B. gossypiperda*, the American variety, 289F. was selected and cage experiments performed in 1929 and 1930, at Khanewal. The plants were grown under muslin cages each 6×6×6 ft. They were given precisely the same agricultural treatments as the crop outside the cages. Infestation within the cages was controlled by spraying or by introducing White-fly adults as desired. One need hardly point out that under cages the plants behave differently than in the open, but comparative results under similar conditions are significant.

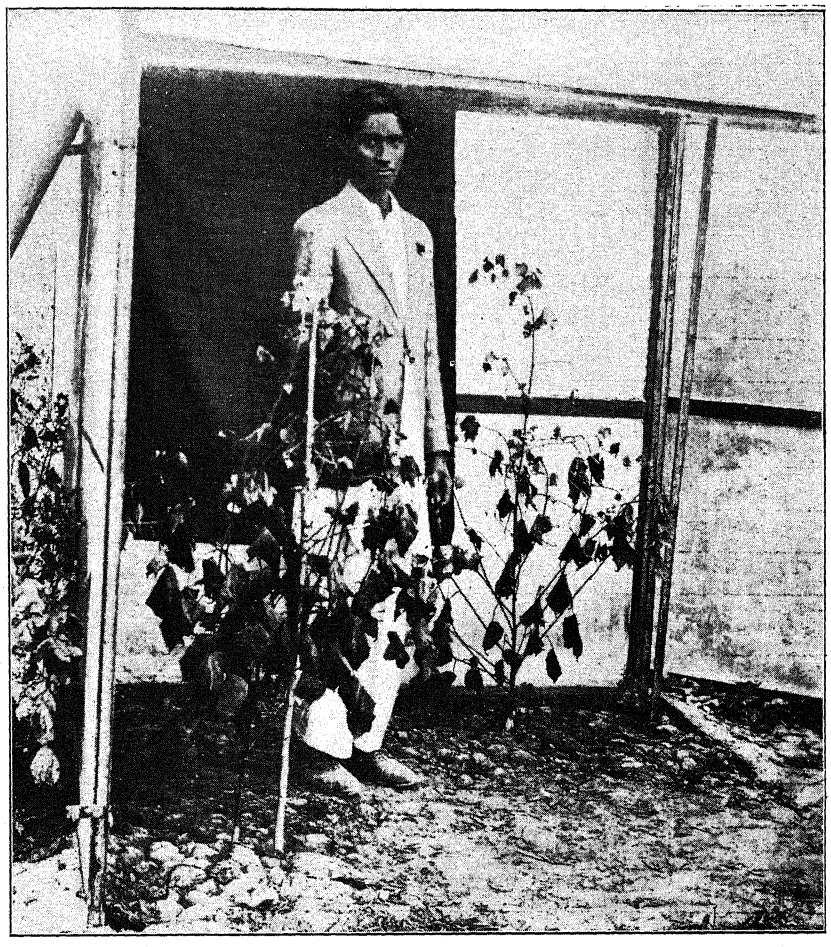
The nature of injury caused by the White-fly has been fully discussed previously by Afzal Husain [1930]. The insect does not produce any visible structural damage to the leaves of the host plant, in other words, there is no spotting, deformation, curling, crinkling or withering. Some of the leaves of the infested plants, as is the case with all infestations by insects that produce honeydew, become oily in appearance and sticky to the touch, particularly on the upper surface, and ultimately on account of the growth of fungus present a sooty appearance. The presence of fungus certainly interferes with photosynthesis and the evil effect of the feeding of innumerable sucking insects can hardly be underrated.

1. Severe infestation.

(*i*) Three cotton plants were enclosed under a cage on 16th June 1929 and numerous adult White-flies were regularly introduced to maintain a uniformly severe infestation. The infestation was very intense and far above that which one finds in nature even in the worst cases. The plants continued growing but the leaves were flaccid and turned black on account of the growth of fungus on the honeydew. No reddening of leaves or premature defoliation was noticed. Only one plant produced five floral buds all of which dropped off.

(*ii*) During 1930, a similar experiment was started on 15th July with two plants instead of three. Premature defoliation was noticed after 8th September 1930 which gave these plants a sickly appearance. In all 189 leaves were produced up to the 8th September, and the maximum size of a leaf determined from the average of three biggest leaves was 8·5 in.×7·1 in. Only three floral buds appeared in this case and all dropped off. The secretion of honeydew and the growth of mould was very marked.

(*iii*) In a duplicate of the above experiment started on the same date in 1930, both the plants grew normally in spite of the severe infestation, but did not produce any floral bud. Premature defoliation was prominent on one of these plants (Plate LVI, fig. 1). The two plants produced a total of 362 leaves and on an average, the maximum size of a leaf calculated from the three biggest leaves was 8·8 in.× 7·8-in. The conditions regarding honeydew and mould were as above.



Cotton plants severely infested by the White-fly (in cage).

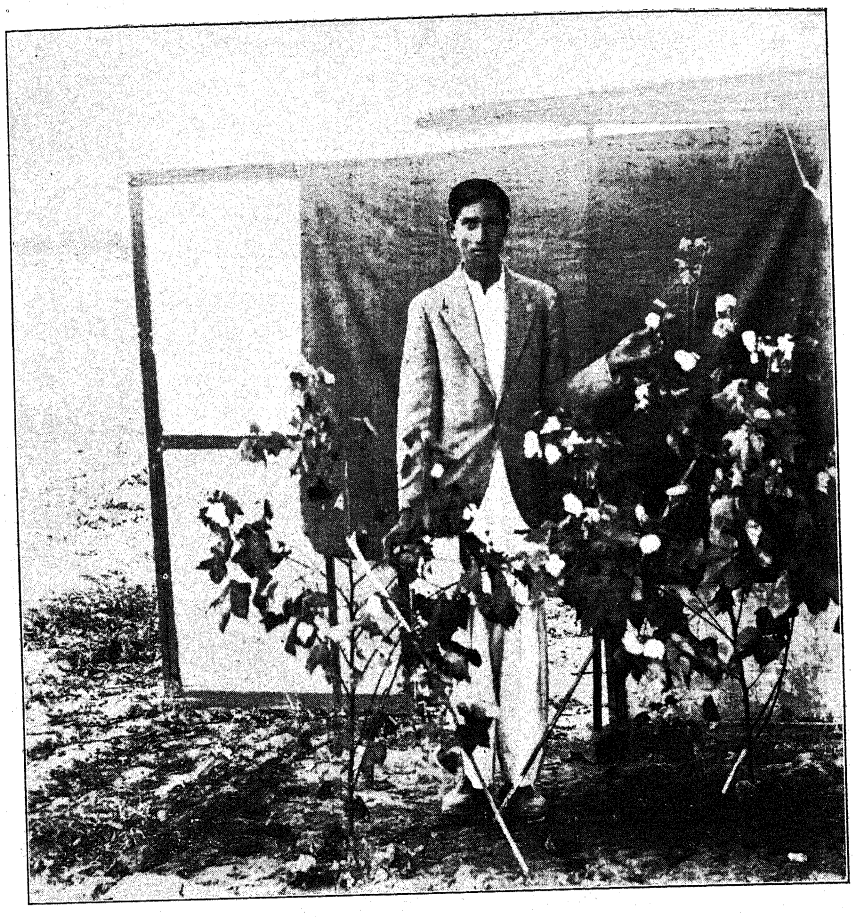


Fig. 1.—Cotton plants with normal infestation of the White-fly (in cage).

(*iv and v*) Similar experiments were started on 18th August 1929 and 1st August 1930. The vegetative growth continued to be normal in both cases whereas the bottom and middle leaves turned black on account of the mould. During 1930, these plants produced 235 leaves and on an average, the maximum size of a leaf calculated on the basis of three biggest leaves was 8·6 in.×6·5 in. In 1929, out of 136 floral buds produced on these plants, only 8 matured giving 94 per cent. shedding, while in 1930, out of 44 floral buds produced, 68 per cent. were shed.

2. Moderate infestation.

(*i*) Two cotton plants were enclosed under a cage on 8th July 1929 and the infestation found under field condition was allowed to continue. By the end of August the bottom and middle leaves had turned black on account of the mould. Out of 601 floral buds produced on these plants, 71 per cent. were shed and 47 per cent. of the rest opened well.

(*ii*) In 1930, similar experiment was started on 15th July. Vegetative growth was normal but the bottom leaves dropped off and a few leaves turned red by the middle of October. These plants produced 364 leaves up to the 8th of September and on an average, the maximum size of a leaf, calculated on the basis of three biggest leaves, was 8·8 in.×6·3 in. Out of 97 floral buds produced, 45 per cent. were shed and 70 per cent. of the rest opened well (Plate LVII, figs. 1, 2).

(*iii*) The above experiment was duplicated on the same date in 1930 and the plants behaved practically in the same manner. In all 309 leaves were produced and on an average, the maximum size of a leaf, calculated on the basis of three biggest leaves, was 10·0 in.×7·7 in. Only 94 floral buds appeared, of which 35 per cent. were shed and 70 per cent. of the remainder opened well.

(*iv and v*) Similar experiments as the above were repeated on 1st and 15th August 1930. The plants maintained quite normal growth. Out of 88 and 89 floral buds produced respectively, only 60 per cent. matured of which 71 per cent. had a good opening.

3. Practically free from infestation.

(*i*) Two cotton plants were enclosed under a cage on 28th June 1929. They were sprayed with rosin compound shortly before being caged and spraying was repeated on 10th August and 23rd August to keep the plants free from attack. In all 537 floral buds were produced of which 60 per cent. were shed and 89 per cent. of the matured bolls opened nicely. The plants on the whole maintained a very healthy condition.

(*ii*) Similarly in 1930, two plants were sprayed with rosin compound on 15th July and enclosed under a cage. They were again sprayed on 25th July after which the surviving insects were hand-picked. The plants in general exhibited a luxuriant

vegetative growth. In all 354 leaves were produced and on an average, the maximum size of a leaf, calculated from three biggest leaves, was 10 in.×8 in. Out of 179 floral buds produced in this case, 28 per cent. were shed. Matured bolls were well developed and 92 per cent. of them opened well.

(*iii*) The above experiment was duplicated on the same date in 1930. The plants grew very tall but there was an indication of premature reddening of leaves and the bottom leaves dropped off. In all 338 leaves were produced on these plants and on an average, the maximum size attained by a leaf, calculated from three biggest leaves, was 10 in.×8 in. Out of 195 floral buds produced 25 per cent. were shed and 94 per cent. of the matured bolls opened well (Plate LVIII, figs. 1, 2).

(*iv and v*) The foregoing experiment was repeated on 14th August during 1929 and on 1st and 15th August 1930. The vegetative growth in all these cases was very good, but in 1929, premature reddening was observed. Shedding of buds and bolls was estimated at 65 per cent. during 1929 whereas, in 1930, it was only 25 per cent. and 28 per cent. respectively, with a corresponding good opening of 88 per cent. and 86 per cent.

It was observed during 1930, that the number of floral buds formed on all the plants under cages was comparatively much below that obtained in 1929. This fall in number was possibly the result of soil conditions because in 1930 the soil on which cages were fixed was of poor quality and moreover the cotton crop was sown after wheat. As all the experiments were performed in the same field, in that particular year, the results are comparable.

TABLE XIV.

Influence of B. gossypiperda *infestation on cotton plants under cages, 1929-30.*

Degree of infestation	Date of starting the experiment	Number of plant	Total number of floral buds, flowers and bolls		Percentage of shedding	Total number of bolls matured	Bolls opened		Percentage of bad opening	Remarks	
			Produced	Shed			Good	Bad			
Free from infestation	28th June 1929	A	246	154	63	92	83	9	10	Plants tall and bushy, leaves green. Premature reddening of leaves.	
		B	291	166	57	125	109	16	13		
	14th August 1929	A	180	125	69	55	44	11	20		
		B	107	64	60	43	32	11	26		
	15th July 1930	A	62	19	31	43	38	5	12	Growth luxuriant, foliage green, indication of the premature reddening of leaves.	Average number of branches per plant 23·5.
		B	117	32	27	85	80	5	6		
	15th July 1930	A	93	26	29	67	63	4	6		
		B	102	23	23	79	74	5	6		
	1st August 1930	A	64	17	27	47	41	6	13	Infestation was removed in the month of August.	Average number of leaves per plant 174.
		B	106	26	25	80	71	9	11		
	15th August 1930	A	145	41	28	104	90	14	13		
		B	54	15	28	39	34	5	13		

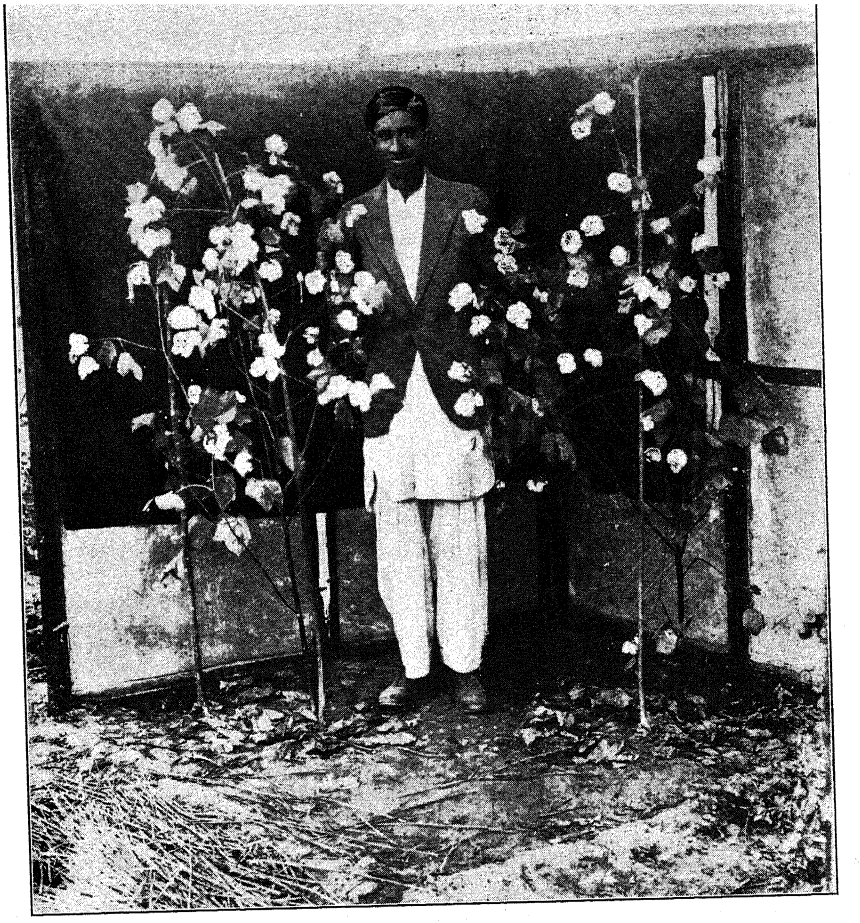


Fig. 1.—Cotton plants kept free from White-fly attack by spraying (in cage).

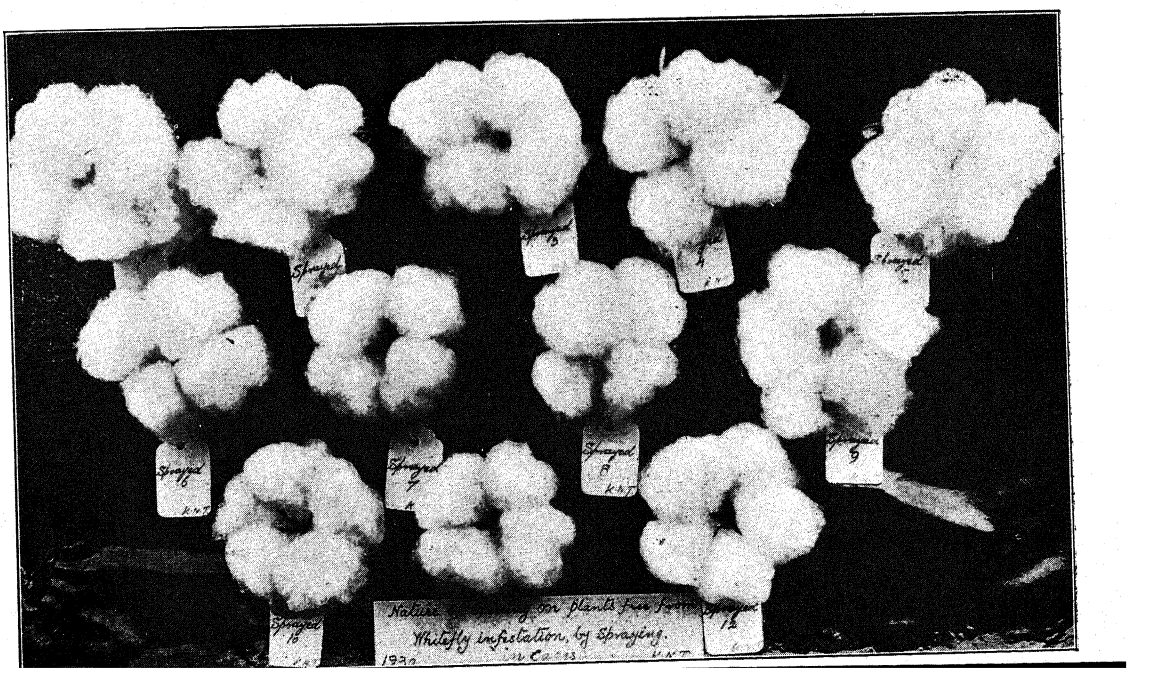


TABLE XIV—*contd.*

Degree of Infestation	Date of starting the experiment	Number of plant	Total number of floral buds, flowers and bolls		Percentage of shedding	Total number of bolls matured	Bolls opened		Percentage of bad opening	Remarks	
			Produced	Shed			Good	Bad			
Moderate infestation	8th July 1929	A	255	176	69	79	37	42	53	Growth quite normal, bottom and middle leaves turned black.	
		B	346	249	72	97	45	52	54		
	15th July 1930	A	37	19	51	18	12	6	33	Plants quite normal. Bottom leaves turned black and were soon shed.	Average number of branches per plant 19·1. Average number of leaves per plant 146·6.
		B	60	25	42	35	25	10	28		
	15th July 1930	A	57	24	42	33	23	10	30		
		B	37	9	24	28	20	8	29		
	1st August 1930	A	49	16	33	33	24	9	27		
		B	39	19	49	20	14	6	30		
	15th August 1930	A	46	23	50	23	17	6	26		
		B	43	12	28	31	21	10	32		
Severe infestation	16th June 1929	A	5	5	100	..	..	..	..	Growth moderately good, leaves turned black and drooping.	
		B	..	..	..	..	..	..	..		
		C	..	..	..	..	..	..	..		
	18th August 1929	A	83	78	94	5	..	5	100	Premature defoliation was noticed.	
		B	53	50	94	3	..	3	100		
	15th July 1930	A	..	..	..	..	..	..	..	Premature defoliation, plants appeared sickly.	Average number of branches per plant 17·5.
		B	3	3	100	..	..	..	..		
	15th July 1930	A	..	..	..	..	..	..	..	Plants normal.	Average number of leaves per plant 119·7.
		B	..	..	..	..	..	..	..		
	1st August 1930	A	10	6	60	4	3	1	25	Infestation started late.	
		B	34	24	71	10	7	3	30		

The cage experiments mentioned above indicate that, as a result of White-fly infestation, the vegetative growth under normal conditions of attack, is hardly affected. But, on the other hand, the reproductive activities of the plant are severely interfered with. The White-fly infestation could not be held directly responsible for reddening of leaves because this character developed even on those plants which were thoroughly sprayed and were thus practically free from this insect. On the contrary, the plants which were severely infested, were absolutely free from this symptom. Premature defoliation was not common in the fields where soil was good, and normal care was taken as regards irrigation. On the other hand, in poor soils with poor irrigation, premature defoliation was rather common. The White-fly in such cases might be a minor contributing factor. Only in cases of extreme infestation (cage experiments), defoliation was noticed in cases where the attack had started very early and a severe intensity of infestation was maintained throughout the season. Thus, under moderate infestation the attacked plants continue to grow and maintain a fairly healthy condition.

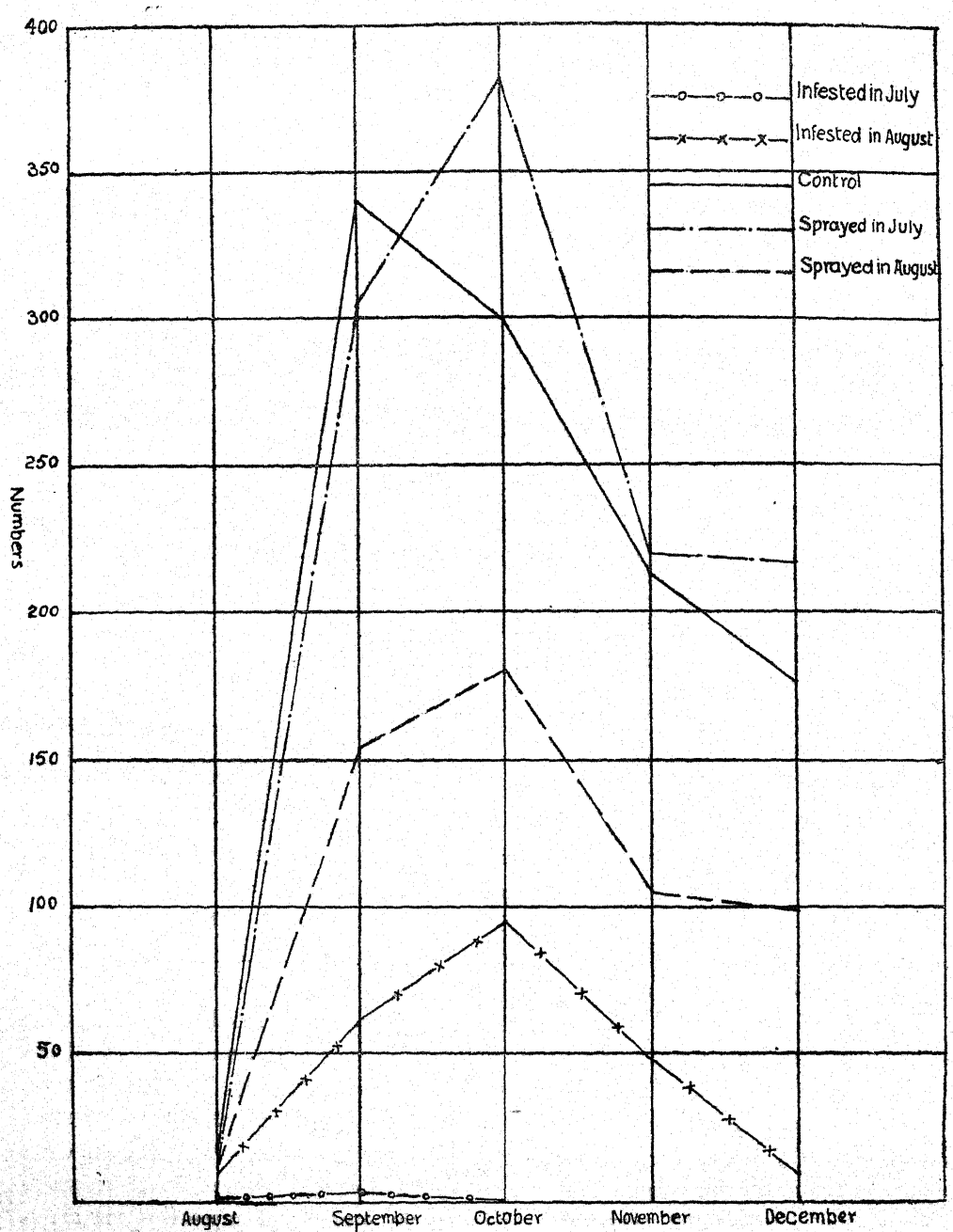


Fig. 4.—Number of surviving buds, flowers and bolls on caged cotton plants at the end of each month, 1929.

The sprayed plants exhibited an exceedingly good vegetative and reproductive growth. The plants became bushy with the foliage quite green, bolling was profuse and the opening quite good. In cases of severe infestation, the development of floral buds had almost totally stopped and even those buds which had actually appeared were all shed (Figs. 4 and 5). In cases of moderate attack, or when severe attack had started late in the season, the number of matured bolls on the plants was appreciably reduced, the percentage of boll-shedding was comparatively very high, and at the same time, the opening was considerably poor (Table XIV). In other words, the bud and boll formation was indirectly proportional, while bud and boll-shedding and bad opening of the bolls were directly proportional to the intensity of the White-fly attack.

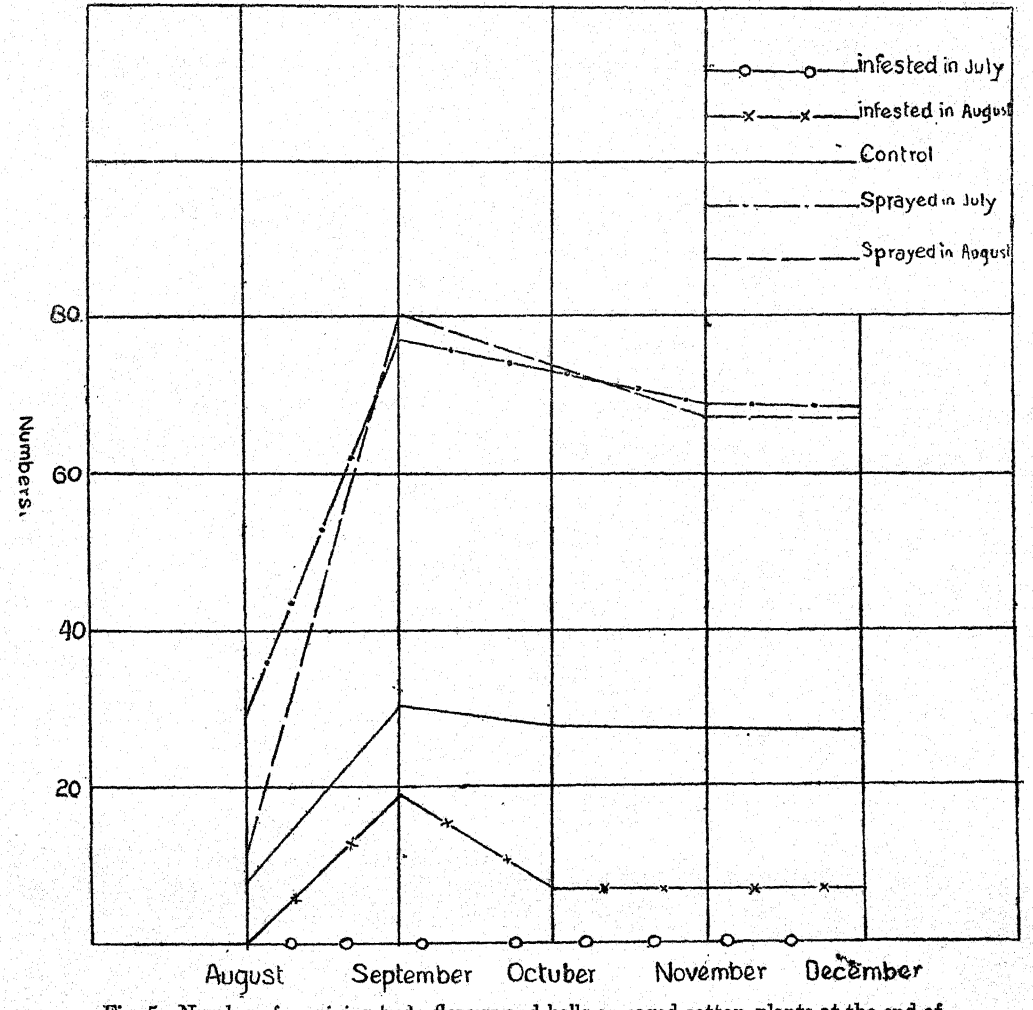


Fig. 5—Number of surviving buds, flowers and bolls on caged cotton plants at the end of each month, 1930.

A series of observations were also made on the number and weight of seeds per boll from sprayed and unsprayed plants under cages. It was estimated that the sprayed plants gave a higher number of seeds per boll and also higher lint percentage than those unsprayed. It is quite evident from the above experiments that, with moderate infestation, the vegetative growth of the plants is not materially affected but it is the reproductive faculty which is interfered with considerably.

It is necessary here to make a distinction between this condition and the condition of the plant during years of general failure. The plants attacked by White-fly would not produce bolls while according to Roberts [1929], during years of failure there would be a fairly large number of bolls but these would not open properly.

It appears that the White-fly, by sucking cell sap, interferes with the metabolism of the plants and upsets the normal behaviour in so far as reproduction is concerned. Attempts are being made to analyse these factors.

Boll-shedding and bad opening is undoubtedly a result of malnutrition, but this malnutrition, which is the result of a severe infestation of the White-fly, would be expressed more prominently in the non-formation of flowers and consequently of bolls. It is most unlikely that, as a result of White-fly infestation, there should be profuse bolling as stated by Roberts [1929] and then the bolls would shed and *tarek* appear.

Parasites.

B. gossypiperda nymphs in the 3rd instar and pupae, have been found parasitized by chalcid parasites (not identified yet). The parasites deposit their eggs within the body of the host. The adults emerge out of the pupal skin by cutting circular holes. Laboratory experiments carried out at Khanewal, indicated that the duration of the life-cycle of the parasite is six or seven days during the month of August (Plate LIX, figs. 1-4).

Parasitization in nature was noticed to be very low at first, *i.e.*, in June and July but with the advance of season it increased, reaching an average of 23·6 per cent. in September (1929). The parasite, however, does not effect a satisfactory control. The extent of parasitization during different months of the year has been determined by actual counts of the attacked pupae cases.

LIFE-HISTORY OF PARASITE.

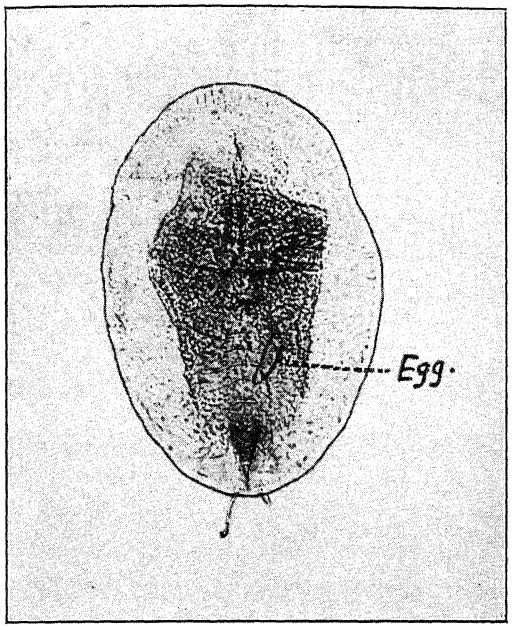


Fig. 1.—Egg laid within the body of White-fly nymph.

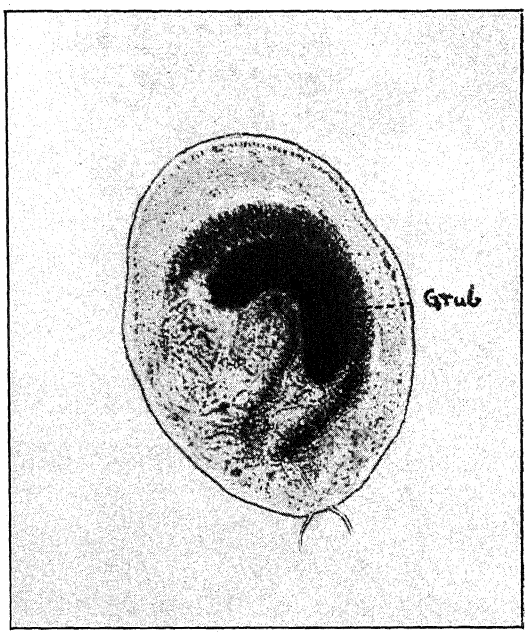


Fig. 2.—Parasite grub in the body of the nymph.

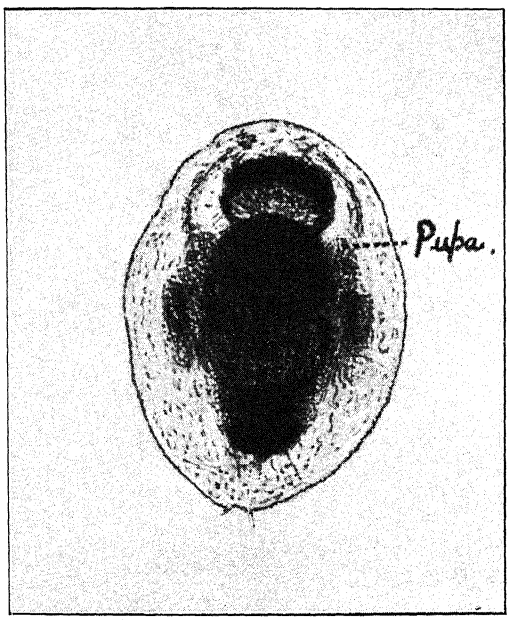


Fig. 3.—Parasite pupa.

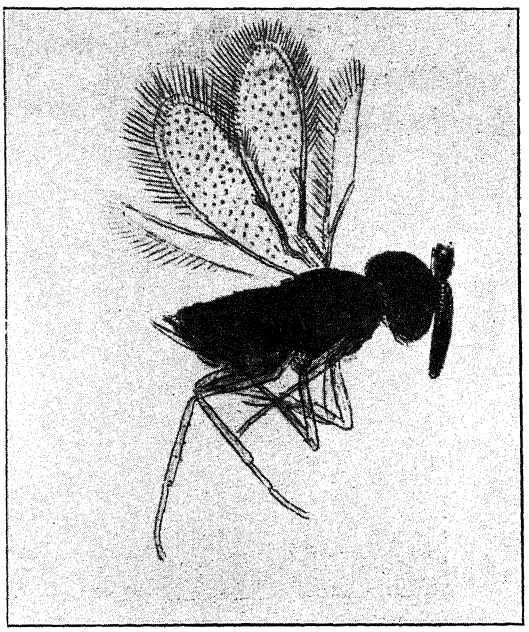


Fig. 4.—Adult parasite.

TABLE XV.

Parasitization in nature (1929, 1930).

Month	1929			1930			Remarks
	No. of pupae cases examined	No. of pupae cases parasitized	Percentage of parasitization	No. of pupae cases examined	No. of pupae cases parasitized	Percentage of parasitization	
June	272	7	2·5	280	5	1·7	
July	...	...	...	756	36	4·7	
August	470	84	17·8	1,481	176	11·8	
September	1,865	442	23·6	1,405	269	19·1	In certain individual cases, maximum parasitization was as high as 33 per cent. during September, 1929.
October	610	105	17·2	942	162	17·1	
November	1,642	80	4·8	1,437	187	13·1	

PREDATORS.

Larvæ of a lacewing fly (*Chrysopa* sp.) and of a coccinellid beetle (*Brumus* sp.) have been observed feeding on the adults of the cotton White-fly. Further, it has also been observed that the number of adults killed by an individual grub of *Brumus* or *Chrysopa* sp. far exceeds that which is actually required for its feeding. Judging from their activity in nature, it is, however, quite obvious that these insects do not afford a good check. A few casual observations made to estimate the population of both these predators in nature, are given in the following table :—

TABLE XVI.

Population of the predators in nature.

Date	No. of plants examined	*Brumus* sp.		*Chrysopa* sp.		Remarks
		Population calculated per 100 plants		Population calculated per 100 plants		
		Adults	Grubs	Adults	Grubs	
1929.						
31st August	25	52	212	396	1,084	
5th September	25	348	404	321	1,632	
24th ,,	25	...	...	...	380	
3rd October	25	128	...	108	240	
13th ,,	25	104	48	60	52	
20th ,,	25	...	95*	...	56	*Observations were taken on 100 plants instead of 25.
1st November	25	...	...	60	...	

Control measures.

Insecticidal.

Preliminary experiments to determine the efficacy of the insecticidal methods of control were carried out in 1929 [Afzal Husain, 1930]. Spraying with rosin compound and fish oil soap gave encouraging results. In 1930 the following scheme of spraying was adopted, and rosin compound was employed :—

I. One spraying.—July, August or September.

II. Two sprayings.—July and August, July and September, or August and September.

III. Three sprayings.—July, August and September.

IV. Fortnightly sprayings.—July to September.

The plots used in all the foregoing experiments were $\frac{1}{20}$th of an acre each, but they were further sub-divided into outer non-experimental and inner experimental areas. Thus each of the experimental plots measured about $\frac{1}{40}$th of an acre. Yields from the experimental plots were compared with those from the corresponding area in the unsprayed plots. The crop was sown on ridges and the number of plants in both the control and treated plots was approximately the same.

It was observed that the sprayed plots maintained a much healthier condition and that the opening of bolls was also comparatively better than in the case of the unsprayed plots. Further, the effect of spraying lasted for a considerable time and the intensity of attack on the sprayed plants was comparatively low throughout the season.

Comparative infestation in these experimental plots was observed to be the lowest in those plots which were sprayed both in July and August and the yields obtained from these plots were the highest —3 maunds, 22 seers per acre above control (Table XVII).

From the results obtained it appears that a single spraying in the month of August or one spraying in July and one in August, are very beneficial (Plate LX, figs. 1 and 2). As this experiment has been performed on very small areas we are not prepared to make definite statement regarding the efficacy or economics of the method, nor do we offer, at this stage, any explanation of the comparative results obtained by two sprayings in July and August, July and September and August and September. We must await further large scale trials before a precise statement regarding the value of spraying, and the most economic method and best time for spraying, can be made. The average cost of spraying with rosin compound was worked out by the British Cotton Growing Association (Punjab) Limited, Khanewal, at Rs. 2-9 per acre. The estimate was based on the figures obtained by spraying 365 acres in 1930 [Thomas, 1932].

Fig. 1.—Condition of cotton crop in a plot with normal infestation of White-fly (control plot).

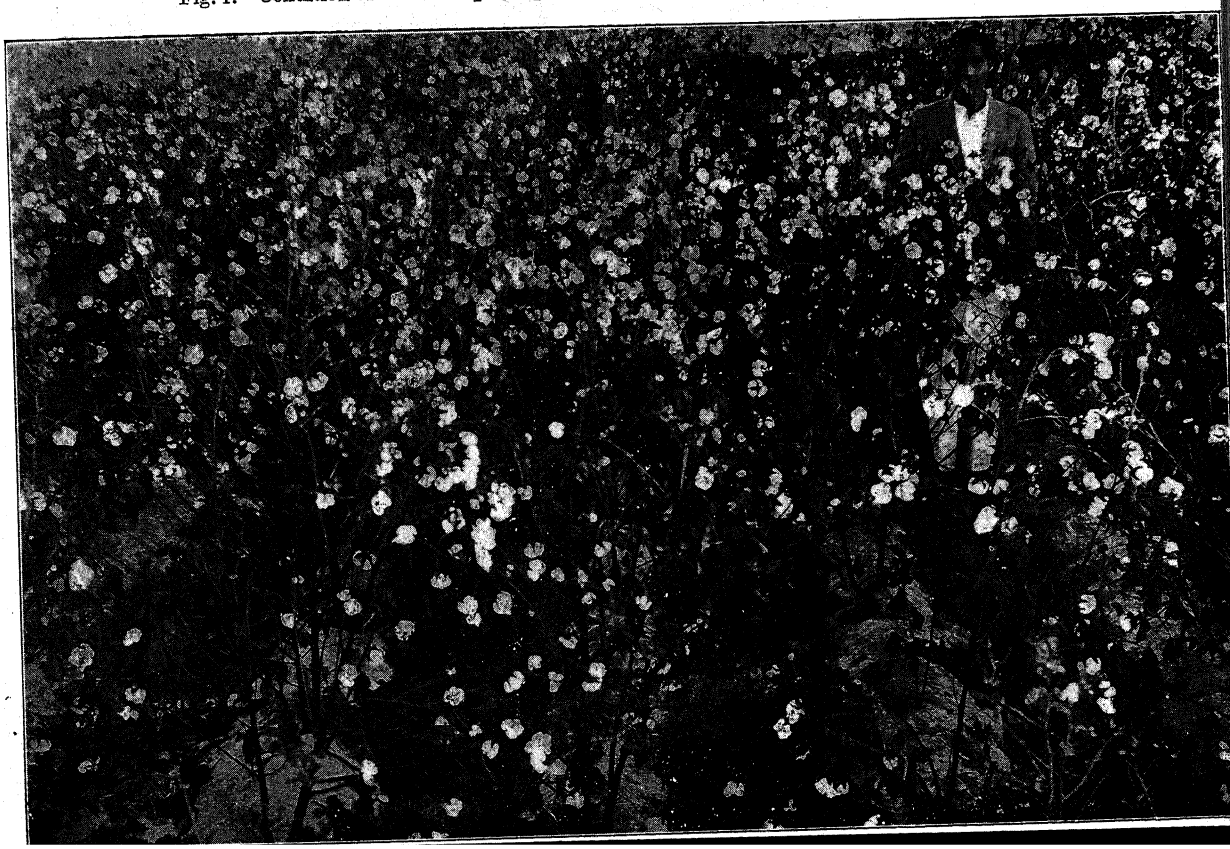


TABLE XVII.

Yields obtained from sprayed and unsprayed experimental plots (1930).

Sprayed in the month of	Line No.	Yield from sprayed plots	Yield from unsprayed (control) plots	Increase in yield by spraying	Average increase per acre	Remarks
		Seers.	Seers.	Seers.	Mds. Srs.	
SINGLE SPRAYING						
July	I	21·25	17·62	3·63		
	II	36·37	28·25	8·12	1 35	Each experiment was replicated thrice in each line of the square. There were, therefore, 12 replications in all.
	III	24·75	18·50	6·25		
	IV	20·25	15·62	4·63		
August	I	27·06	17·62	9·44		
	II	39·00	28·25	10·75	2 38	
	III	25·75	18·50	7·25		
	IV	23·62	15·62	8·00		
September	I	20·75	17·62	3·13		Each experimental plot was $\frac{1}{10}$th acre.
	II	31·81	28·25	3·56	1 4	
	III	21·50	18·50	3·00		
	IV	19·31	15·62	3·69		
DOUBLE SPRAYING						
July and August	I	24·94	11·12	13·82		
	II	40·06	27·62	12·44	3 22	*Four replications.
	III	*32·06	23·25	8·81		
	IV	28·87	19·12	9·75		
July and September	I	24·37	11·12	13·25		
	II	37·00	27·62	9·38	2 39	
	III	26·56	18·81	7·75		
	IV	24·44	19·12	5·32		
August and September	I	20·56	11·12	9·44		
	II	30·75	27·62	3·13	1 34	†Five replications.
	III	32·81†	23·25	9·56		
	IV	22·68	19·12	3·56		
TRIPLE SPRAYING						
July, August and September	I	24·94	14·00	10·94		
	II	34·25	26·56	7·69	2 30	
	III	25·19	17·75	7·44		
	IV	21·19	14·31	6·88		
FORTNIGHTLY SPRAYING						
Sprayed fortnightly from July to September.	I	20·56	12·94	7·62	2 22	

SUMMARY.

The life-history of *Bemisia gossypiperda* has been studied and its various stages described. There may be twelve generations in a year but the broods overlap. The eggs are laid singly on the underside of leaves, the maximum number of eggs laid by a female in 18 days was 119. The incubation period extends from 3 to 33 days. The duration of the nymphal stage varies from 9 to 81 days, the pupal period lasts from 2 to 8 days. The average duration of life-cycle from April to September is 17·5 days. The longest life-cycle extends to 107 days between November and February. The adults, as a rule, emerge during the day time and are short-lived during summer. The progeny from parthenogenitic eggs consists of males only.

Infestation, as a rule, is severest on cotton during July and August. The white-flies do not show any particular preference for different varieties of cotton. The incidence of attack on the early sown crop was comparatively higher upto September, after which it was practically uniform on all the sowings. There is not much difference between the cotton sown between 1st May and 1st June but the cotton sown later escapes the attack of the pest.

The intensity of attack was higher on the manured plants but plants treated with soda nitrate at one maund per acre, showed comparatively less attack.

The normal infestation of White-fly does not very much interfere with the vegetative growth, but the development of floral buds is hindered, and the shedding percentage increases. Shedding and bad opening of bolls are directly proportional to the intensity of attack.

Parasitization in nature was observed to reach a maximum of 33 per cent. of the pupæ during September. Some predators have also been noticed.

Over three dozen different plants including weeds and cultivated crops have so far been recorded as alternative host plants of the cotton White-fly. Cross inoculation gave successful results in case of a number of alternative food plants.

Spraying of cotton fields with rosin compound once in the month of August or twice, first in July and again in August, has given very encouraging results.

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STUDIES IN INDIAN OATS

I. THE IMPROVEMENT OF THE CROP BY SELECTION AND THE ACCLIMATIZATION OF EXOTIC TYPES.

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(With two text-figures).

I. Introduction.

The popularity of oats as a fodder crop is gradually increasing in India and it is not uncommon now to find large areas of this crop especially near towns and military stations. The local ryot too has begun taking it up to some extent for green fodder as well as for its seed. The grain in this country is mostly used for feeding horses and the straw is recognized as being more nutritious than that of wheat or rice. As an article for human consumption the oat grain has not yet attracted any attention in India. This is perhaps due to the fact that the oat which is ordinarily cultivated here has a very thin kernel and is rather difficult to husk out. The present paper is intended as an introduction to the more detailed study of the oat crop which forms the subject of subsequent papers in this series. The second paper [Shaw and Bose, 1933] deals with the inheritance of characters in some interspecific crosses between Indian and exotic oats and will lead on to an investigation of the economic possibilities of the hybrids.

Oats belong to the genus *Avena* of the Natural Order *Gramineae*. Watt [1908] has reported the existence of 13 species of *Avena* in addition to the cultivated one met with in India. The wild forms all occur on the Himalayas and none of the truly indigenous species are ever cultivated. In all books of references the commonly cultivated species in this crop has till now been called *Avena sativa* Linn., the species which is common in the United Kingdom. A careful study of this crop as grown in Bengal, Bihar and Orissa, United Provinces of Agra and Oudh and

Punjab has definitely shown that the Indian oat belongs to the cultivated species of *Avena sterilis, culta* Linn. [Bose, 1929]. It may be noted that this is the species generally grown in the warm countries round about the Mediterranean, and in all tropical and sub-tropical countries, and differs but slightly from *A. sativa* which is commonly cultivated in more temperate climates. Lately some American workers have adopted the use of the name *A. byzantina* Koch. for *Avena sterilis* var. *culta* Linn. and this synonym is gaining in popularity. The main differences between the two species of *Avena* are presented in the following table :—

TABLE I.

Characteristic differences between the two main cultivated species of oats.

Character.	*A. sativa* L.	*A. sterilis* L. var. *culta* or *A. byzantina* Koch.
1. Form of attachment of the lower grain with the main axis.	Not articulated	Articulated. Shows a "Sucker-mouth" scar at the base of the lower grain.
2. Form of attachment of the upper grain.	Articulated. When pulled, it breaks off from the rachilla which remains attached to the lower grain.	Not articulated. When pulled, it comes off carrying the rachilla with it.
3. Awns	Long, coarse and frequently geniculate and twisted at the base.	Thin and practically straight.
4. Basal hairs	Very few fine hairs at the base of the lower grain, sometimes even nil.	Many prominent hairs at the base of the lower grain.

In the 'Ain-i-Akbari', 1590, mention is made of oats in the chapter on fodder, but leaving this aside the cultivation of this crop in India can certainly be traced to the beginning of the 19th century. No separate statistics of area or production are maintained and the foreign trade is normally small in comparison to that of other grains produced in the country [Cotton, 1919].

The young oat plant in India belonging to the species *A. sterilis*, is sub-erect. The roots are shallow, fibrous and numerous. The culm or stem consists of a hollow cylinder about 0·5 cm. in diameter, intersected by a number of nodes usually 3 to 5 from which the leaves arise, terminating in the inflorescence. The first node from the top is generally hairy. The ligule is well developed. The leaves are long and narrow, tapering gradually to the apex, with a drooping habit and yellowish green colour. There are a few fine short hairs on the margins. The panicle is erect, spreading and equilateral. The spikelets are generally 2- and rarely 3-grained, the third flower in the spikelet usually remaining abortive. The basal articulation

of the lower grain is very marked, leaving a 'sucker-mouth' scar when the grain is separated from the main rachis. The upper grain, however, always breaks off with the rachilla. Glumes about 25 mm. long, pointed, having 9 coarse nerves. Awns thin, sometimes twisted at the base in a clockwise direction, but never geniculate. Lower grains always awned but upper grains rarely so. Basal pubescence very prominent and consisting of numerous brownish hairs. Grain yellowish, the lower one about 20 mm. long, 3 mm. wide and 2 mm. thick. Kernel hairy, about 10 mm. long. Stooling capacity medium to sparse. Maturity early.

Season and cultivation.—Oats are generally sown in India in the *rabi*, somewhere in October or November and are harvested by the following March or April.

In parts of the country subject to heavy rainfall, oats grow best on well-drained friable soils of a fair depth whilst in other parts, with a lower rainfall, they are frequently grown on soils which are suitable for wheat and barley. The tillage and other operations required for this crop are likewise similar to those demanded by wheat and barley. The seed rate generally varies from 70 to 100 lb. per acre, depending on the variety, the environment and on the time of sowing. High tillering varities need not be sown as heavily as varieties with a smaller tillering habit. Duthie and Fuller [1892] have reported an average yield of 18 maunds on irrigaged and 10 maunds on unirrigated land per acre, but yields ranging from 25 to 35 maunds to the acre are now being obtained at Pusa on unirrigated land with improved strains.

Chromosome numbers.—The haploid number of chromosomes in *Avena sativa*, *A. sterilis*, and *A. byzantina* has been reported to be 21 by Kihara [1919]. Huskins [1926] found 21 in *A. sterilis*. Aase and Powers [1926] report a haploid number of 21 in *A. sativa*, and Goulden [1926] also found *A. sativa*, *A. sterilis* and *A. fatua* to have 21. The same number was observed also in a preliminary study made at Pusa with Scotch Potato oats (*A. sativa*) and B. S. 1 oats (*A. sterilis*).

II. Improvement of the crop.

The improvement of a field crop such as oats, with a view to the production of a variety superior in quality and yielding power to the indigenous crop, necessitates as the first step the isolation by selection of the types already existing in the mixed crops present in the country. If the desired aim cannot be achieved by selection, resort must be made to the introduction and acclimatization of exotic forms and possibly to hybridization between these and the types which are endemic to the country. All three methods have been used by us in our study of the improvement of this crop.

(*1*) *Selection.*—A number of pure line selections was made from Bihar oats which were supplemented by selections from samples from various parts of Northern

India. As already explained all the selections were found to belong to the species of *Avena sterilis, culta* L., and were distinguished from one another only by time of maturity and yielding power.

A preliminary test of nine selections in comparison with a standard variety, the Meerut oat, indicated that three of these selections were of higher yielding power. Subsequent trials narrowed the choice to two types which are now distributed under the numbers B. S. 1 and B. S. 2.

Description of types.—B. S. 1 and B. S. 2. oats are thus two pure-line selections evolved at Pusa from Bihar oats which proved superior by virtue of their resistance to drought and diseases, their earliness, high yield and quality. In the early stages the plants of both these types are semi-erect in habit and have long and fairly narrow leaves, with a very few marginal hairs. Tillering capacity is moderate; leaf-sheath is glabrous and has a deep purple tinge at the base. The straw is long and fine and is rather weak and liable to lodging. The panicle is medium in size, equilateral, more or less erect. Branches somewhat long and pendulous. Spikelet long with two grains; glumes long, thin and translucent, yellowish green in colour with nine prominently marked veins. Awns thin and slightly twisted at the base, always present on the lower and occasionally on the second floret. Many short hairs on the callus but none on the palea. Grain dirty yellow with a greyish colour on the tips of the glumes, and usually long. The weight of straw is about one and a half times to twice the weight of grain depending, of course, on the type of oat and its relation to the soil and weather conditions prevailing. Table II shows the yields of grain and dry straw (*bhusa*) obtained from B. S. 1 and B. S. 2 in different years, as well as the ratio of grain weight to straw weight.

TABLE II.

Yields of grain and straw (bhusa) *and their ratios in B. S. 1 and B. S. 2 oats.*

Year	Area of plot in acres	B. S. 1 oats			B. S. 2 oats		
		Yields in lbs.		Ratio of yields—grain to straw	Yields in lbs.		Ratio of yields—grain to straw
		Grain	Straw		Grain	Straw	
1927	0·20	617	794	1 : 1·29	557	746	1 : 1·34
1928	0·20	805	1244	1 : 1·54	—	—	—
1929	1·57	3733	6603	1 : 1·77	4145	7003	1 : 1·69
1933	0·20	490	946	1 : 1·93	363	624	1 : 1·72

Both are early in maturity and as a matter of fact the difference between B. S. 1 and B. S. 2 lies in the time of maturity. The former is about one or two weeks earlier than the latter. At places where comparatively large areas are to be put under oats it seems to be an advantage to grow both these types simultaneously so that the whole area is not ready for harvest at the same date.

(2) *Acclimatization.*—The Indian oat in comparison with the crops in the United Kingdom is generally deficient in bushel weight and strength and quantity of straw. An attempt was therefore made to import some superior types of oats from foreign countries with the object of seeing whether acclimatization could procure a better type suitable for Indian soil and climatic conditions. The introduction and acclimatization of an exotic crop is a matter which usually presents a number of difficulties in India; the chief obstacle to the success of a plant from a more temperate zone being usually the short growing season which is invariably present in all parts of India. Although late-ripening varieties are known to be potentially prolific, they generally fail to produce good results in this country because most of them are dried up by the incidence of west winds which usually set in at the season when these begin to flower.

The Scotch Potato oat has been grown for a number of years at the Pusa Farm and an attempt is being made there to acclimatize this variety to Pusa conditions. Although its growth has been reported to be very promising it has always been very late in flowering and has invariably been damaged by early hot west winds. The yielding power of this oat in Pusa has therefore been low.

The following strains of oats were imported and tried in the Botanical Section at Pusa for a number of years:—

TABLE III.

Variety	Country from where imported
1. Scotch Potato oats	British Isles.
2. Abundance „	
3. White Cluster „	
4. New Harvester „	
5. Victory „	
6. Yielder „	
7. Orion „	
8. Iowa 103 „	United States of America.
9. Iowa 105 „	
10. Iowar 670 „	
11. Kanota „	
12. Gopher „	
13. Nebraska 21 „	
14. Kinwada „	South Africa.

The exotic oats when grown under Pusa conditions were subjected to an environment markedly different to that to which they were habituated and possibly

on account of this almost all of them showed signs of sterility. Five plants from each of the important exotic varieties and also from B.S. 1 were selected at random in 1927. Counts were made of the number of spikelets with one, with two fertile florets and with sterile florets per plant and averages of five plants expressed in percentages were recorded under each heading. The results are depicted in Table IV and show—

(1) that the majority of spikelets in the exotic oats were 1-grained, *i.e.*, only the primary florets were fertile,

(2) that only in the case of the Abundance oat was there an appreciable number of 2-grained spikelets, *i.e.*, spikelets with both the primary and secondary florets fertile,

(3) that all the exotic oats under observation had some sterile spikelets ranging from 7·32 per cent. in the case of Yielder to 62·65 per cent. in the case of the New Harvester, and

(4) that the Pusa selection, B.S. 1, had most of its spikelets double-grained, a very few single-grained and none sterile.

TABLE IV.

Average amount of seed-setting in some oats. Examined on 16th April 1927.

Type of oat	Percentage setting (Average of five plants)			Source of seed
	1-grained spikelets %	2-grained spikelets %	Sterile-spikelets %	
New Harvester	36·62	0·73	62·65	Grown at Pusa from seed imported from England
Victory	43·05	13·95	43·00	,,
Abundance	42·24	32·37	25·39	,,
Scotch Potato	71·30	11·05	17·65	,,
White Cluster	86·84	0·48	12·68	,,
Yielder	86·36	6·32	7·32	,,
B. S. 1	4·50	95·50	0	Pusa selection, grown at Pusa

Most of the exotic oats produced excellent growth and splendid straw but all the varieties excepting Kinwada were invariably too late and failed to set a normal amount of seed. Kinwada oat was comparatively early but the grain was thin and not very promising.

A fair idea about the heavy growth of straw and the small amount of seed setting in some of the exotic oats as compared with Indian oats may be gathered from the following table :—

TABLE V.

Outturn of some exotic and some Indian oats grown in the same field in 1926-27.

Seed	Type of oat	Outturn in lb. per acre		Ratio of outturn of grain to that of straw
		Grain	Straw	
Exotic	Victory	256	6,012	1: 23·48
	Abundance	478	5,910	1: 12·36
	Yielder	461	5,980	1: 12·97
	New Harvester	102	5,090	1: 49·91
	Scotch Potato	375	4,442	1: 11·85
	White Cluster	205	3,520	1: 17·17
Indian	1—1	2,634	3,126	1: 1·19
	4—1	2,480	3,024	1: 1·22
	7—1	2,685	3,116	1: 1·16
	7—3	2,532	3,054	1: 1·20
	10—4	2,942	4,325	1: 1·47
	12—1 (B. S. 1)	3,085	3,977	1: 1·29
	14—2	3,013	3,905	1: 1·30
	19—1 (B. S. 2)	2,788	3,731	1: 1·34
	19—3	2,532	3,792	1: 1·50
	Meerut	2,511	3,710	1: 1·48

Owing to the fact that all the exotics under trial in this experiment were very late in maturity a normal amount of seed-formation could not take place in them because of the drying of the late flowers by hot west winds. The ratios of the outturn of grain to those of straw have therefore remained abnormally low in these oats. The earlier maturing Indian oats, all of which ripen almost before the exotics begin to flower, have however shown higher yields of grain and ratios between grain and straw yields which are much nearer equality.

(*3*) *Hybridization.*—The defect shown by the exotic types in growing power and time of maturity under Indian conditions indicated to us that the desirable qualities of these types could only be established in Northern India by hybridization with indigenous forms. With this object a number of crosses have been made between some of the exotics and the B.S. selections, and an account of the genetical results and the economic significance of the hybrids evolved will form the subject of subsequent papers. The fact that these crosses are interspecific, *viz.*, between *A. sativa* L. and *A. sterilis, culta* L., has not hindered the production of fertile hybrids and a number of apparently desirable hybrids have now been fixed.

III. The economic aspect.

As in most other cultivated plants in India, the value of the oat crop depends principally on the yield of grain per acre and to some extent on the yield of straw or even of green fodder per acre.

The yielding power of a variety of oats depends on several factors, such as its power to set seed, habit of growth, time of flowering and disease-resistance.

It has been shown in the foregoing pages that B.S. 1 and B.S. 2 oats have been evolved at Pusa by selection from Bihar oats; that these have proved to set seed of fairly good quality and good straw, the dry weight of which is about one and half times to twice the weight of grain; that India is a country which favours early types owing to the growth period here being rather short and that the B.S. oats have geared themselves well to these conditions owing to their early maturity and drought resistance.

That the spacing given to plants exerts a good deal of influence on the variability of the oat crop even under similar climatic and soil conditions, may be gauged from the graphs depicted below (Figs. 1 and 2). In an experiment conducted in 1929 two adjoining small plots were sown with B.S. 1 oats. The rows were one and a half feet apart and the seed was drilled with a hand plough. Plants in one plot were thinned out and spaced at a distance of one foot apart. In the other plot the plants were not thinned and remained about three to four inches apart.

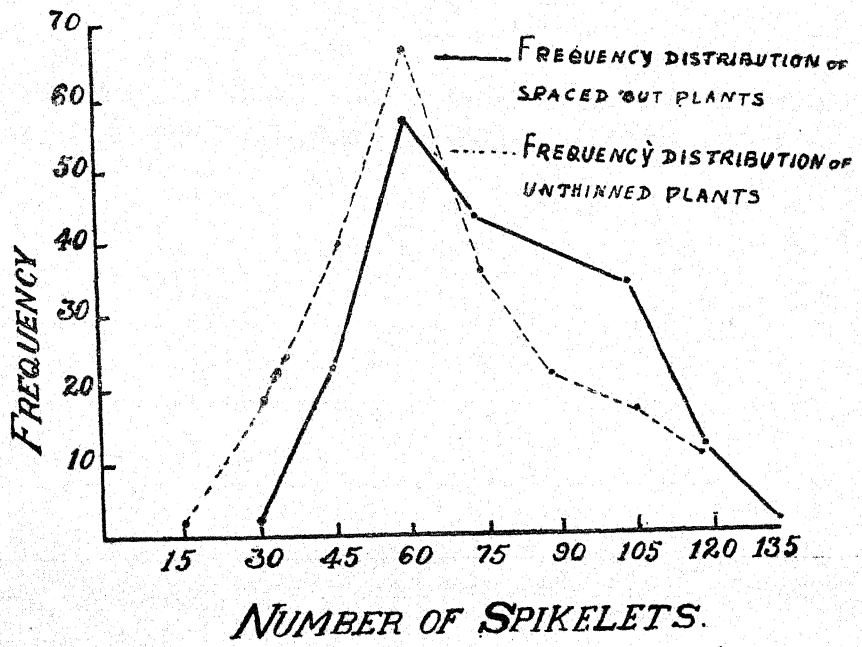

Fig. 1.—Effect of spacing on the average number of spikelets per panicle.

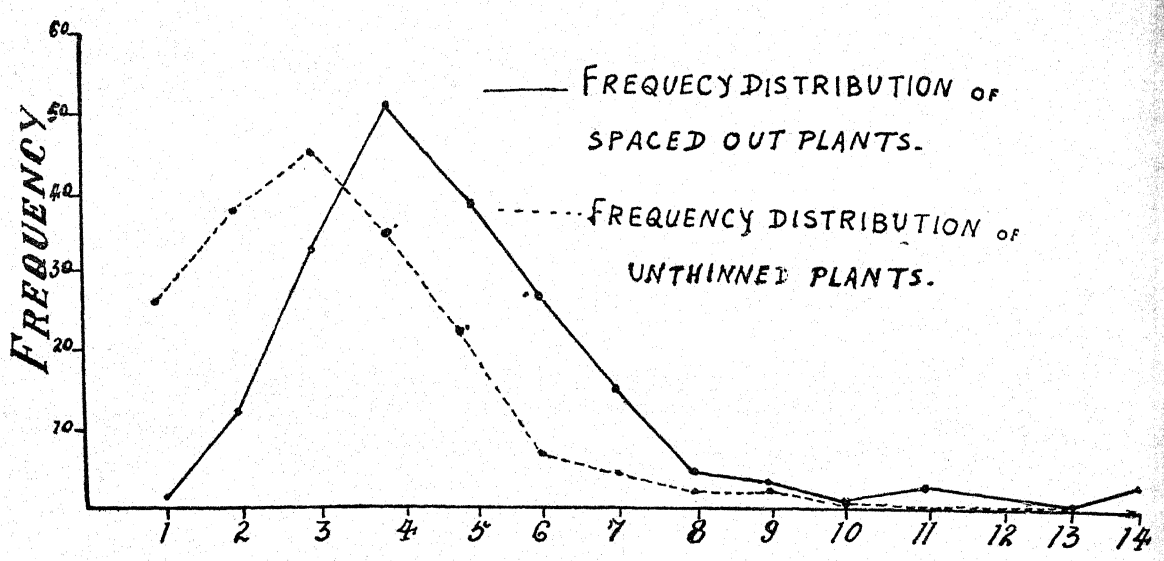

Fig. 2.—Average number of tillers per plant in B.S. 1 oats in 1929.

Fig. 1 shows the effect of spacing on the average number of spikelets per panicle per plant while Fig. 2 represents the same effect on the average number of tillers per plant. The dotted lines show the frequency distribution of the plants in the control, unthinned plot, whereas the heavy lines indicate the distribution of plants in the thinned plots. A marked increase in the number of spikelets per panicle and in the number of tillers per plant has been shown in each case by the plants which were spaced out one foot apart.

The following table shows the average bushel weights of B.S. 1 and B.S. 2 oats as observed during the period 1925-1933.

TABLE VI.

Average bushel weights in lbs. of B.S. 1 and B.S. 2 oats during 1925-1933.

Type	1925	1926	1927	1928	1929	1930	1931	1932	1933	Average of 9 years in lbs.	Coefficient of variability (per cent.)
B. S. 1	35·00	34·50	35·50	35·00	30·00	34·75	35·00	34·75	35·50	34·44±0·38	4·92
B. S. 2	34·50	31·50	34·50	35·50	30·50	34·25	35·50	37·00	36·00	34·14±0·43	5·67

It may be noted that the average weight per bushel of both these oats is about 34 lb. and that with slight differences both are almost similar in density. Seasonal differences on these bushel weights are also apparent. The normal bushel weight of first class oats in the United Kingdom is from 35 to 42 lb. It is evident, therefore, that considerable improvement is possible in this respect in Indian oats and in fact this is one of the directions in which it is hoped to effect improvement by hybridization.

Yields.—B.S. 1 and B.S. 2 have now stood the test of time and have maintained their reputation for high yields, early maturity and drought-resistance. The following table shows the average yields of these two selections under the Botanical Section conditions :—

TABLE VII.

Average yields of B. S. 1 and B. S. 2 oats at the Botanical Section, Pusa, during 1929-1933.

Type	Average yields in lb. per acre					Average of 5 years in lb. per acre	Coefficient of variability per cent.
	1929	1930	1931	1932	1933		
B. S. 1	2,378	2,322	1,871	2,370	2,446	2277·4±62·47	9·1
B. S. 2	2,878	1,870	1,960	2,617	1,815	2228·0±131·09	19·5

It will be seen that the average yield per acre in B.S. 1 oat has shown a coefficient of variability of only 9·1 per cent. during the last five years, whereas B.S. 2 oat has shown a variability of 19·5 per cent. during the same period. Yields of crops are undoubtedly dependent on the climatic and edaphic conditions under which they are grown, but this increased variability in B. S. 2 may also be due to its comparative lateness in maturity which brings about high yields in years of sufficient soil moisture and good weather during and after flowering and seeding, and low yields in rather drier years with early harvests.

The yielding power of the B.S. selections may also be gauged from the yields obtained at the Pusa Farm and other places and from the results of definite yield trials. The following statement shows the yields obtained in different years.

TABLE VIII.

Outturn of Pusa oats as obtained in the Pusa Farm and other places in North India.

Year	Type	Area in acres	Place	Yield per acre, lbs.
1927-28	B. S. 1	8·0	Pusa Farm	1,524
	,,	1·0	Do.	1,599
	B. S. 2	8·0	Do.	1,547
	,,	1·0	Do.	1,484
	Pusa Farm 1	11·0	Do.	1,265
1928-29	B. S. 1	10·0	Do.	2,005
	,,	7·0	Do.	1,397
	B. S. 2	10·0	Do.	1,835
	,,	7·0	Do.	1,465
	Pusa Farm 1	10·0	Do.	1,679
	,,	6·0	Do.	1,327
	B. S. 1	1·0	Sepaya Farm, Bihar	2,312
1929-30	B. S. 1	0·25	Pusa Farm	1,228
	,,	0·25	Do.	1,712
	,,	0·25	Do.	1,384
	,,	0·25	Do.	1,928
	,,	0·25	Do.	2,016
	,,	0·25	Do.	1,448
	,,	87·00	Do.	1,679
	,,	0·33	Sabour, Bihar	934
	,,	0·33	Malda, Bengal	1,107
	B. S. 2	0·33	Malda, Bengal	594
	B. S. 1	11·00	Dholi Estate, Bihar	2,309
	,,	..	Makrera Model Farm, Beawar, Rajputana.	1,066
1930-31	B. S. 1	34·50	Karnal, Punjab	1,041
	,,	15·25	Pusa Farm	1,213
1931-32	B. S. 1	0·115	Do.	2,404
	,,	0·37	Do.	1,851
	B. S. 2	0·115	Do.	1,755
	B. S. 1	14·50	Do. (Plot of low fertility)	824
	B. S. 2	5·00	Do. (,, ,,)	869
	B. S. 1	7·00	Karnal, Punjab	1,891
	,,	0·60	Do.	1,612

In 1927-28 three selections, *viz.*, 12—1 (B.S. 1), 19—1 (B.S. 2) and 14—2 were tried against Pusa Farm oats in the Agricultural Section, in quarter-acre plots replicated eight times each. In the light of more recent knowledge this experiment is open to criticism on account of the size of plots. The results obtained have already been published [Annual Report, 1927-28] and indicated a significant superiority in the yielding power of B.S. 1 (12—1) over that of Pusa Farm oats. A

comparison of yields from paired contiguous plots of B.S. 1 and P.F. 1 oats and a statistical interpretation is given below :—

TABLE IX.

Yield of oats from paired contiguous plots 1927-28.

Yields in lb. per acre		Difference in lb. (d)	Square of difference (d^2)
B. S. 1	P. F. 1		
2234	1255	+ 979	
1757	1585	+ 172	
1068	616	+ 452	
1807	1191	+ 616	
1224	1273	— 49	
1495	1043	+ 452	
1330	1215	+ 115	
		Total Σ+2737	Σ1791715

Mean difference $= \frac{2737}{7} = 391$ lb.

Standard error of difference $= 141{\cdot}6$

$\therefore$ Value of $t = \frac{d}{\text{S. E.}} = \frac{391}{141{\cdot}6} = 2{\cdot}76$

which lies between the 0·05 and 0·02 levels of significance in Fisher's table of t distributions.

The difference of 391 lb. between these two varieties of oats therefore is statistically significant between the 0·05 and 0·02 levels and shows that B.S. 1 oats are superior to the Farm oats in yielding power.

In 1929-30 two regular yield trials on a small and a large scale respectively were laid out in order to study the comparative yielding powers of the B.S. and Pusa Farm oats.

Experiment I.

Randomized block arrangement.

(*Area of each plot 0·035 acres. 10 replications of each type of oat. Botanical Section.*)

The following table shows the yield of these oats per plot :—

Table X.

Yield of oats in Botanical Section, Pusa, 1929-30.

Block	Actual yield per plot in lb.		
	B. S. 1	B. S. 2	P. F. 1
I	75·0	41·0	35·5
II	43·0	33·0	34·0
III	43·0	27·0	34·0
IV	39·0	31·5	39·0
V	46·5	31·0	36·5
VI	43·0	39·5	43·5
VII	37·0	43·0	41·0
VIII	47·5	39·5	40·0
IX	51·5	40·5	41·0
X	50·0	42·0	48·0
Mean yields	47·55	36·80	39·15

TABLE XI.

Analysis of variance.

Due to	Degree of freedom	Sum of squares	Mean square
Blocks	9	586·90	65·211
Varieties	2	548·45	274·225
Errors	18	897·55	49·864
Total	29	2032·90	..

$$\text{Mahalanobis' } x = \frac{\text{Variance}_1}{\text{Variance}_2} = \frac{274{\cdot}225}{49{\cdot}864} = 5{\cdot}499$$

The expected value of x for the 5 per cent. level for $n_1=2$ and $n_2=18$ is 3·55 and at the one per cent. level is 6·013 (from Mahalanobis' Auxiliary Tables for Fisher's z-test). The observed ratio 5·499 being greater than the expected ratio 3·555, the variance between varieties must be considered significant at the 5 per cent. level of significance.

TABLE XII.

Differences of mean yields.

Type	B. S. 1	B. S. 2	Pusa Farm
B. S. 1 .	..	—10·75	—8·40
B. S. 2	+10·75	..	+2·35
Pusa Farm	+8·40	—2·35	..
Mean yields of each type in lb.	47·55	36·80	39·15

Critical difference for $P=0{\cdot}01$ is 9·094 and that for $P=0{\cdot}05$ is 6·64.

It may be concluded therefore that the observed difference of 10·75 lb. between B. S. 1 and B. S. 2 is statistically significant at the one per cent. level of significance and between B. S. 1 and Pusa Farm oats the mean difference of 8·40 lb. is significant at the 5 per cent. level only, both of course in favour of B. S. 1 oats. The

observed mean difference of 2·35 lb. between Pusa Farm and B. S. 2, on the other hand, is not statistically significant.

Experiment II.

Balanced strip method.

(Area of each plot 0·5 acre. 19 replications of each type. Pusa Farm.)

The table given below shows the yields of paired plots under B. S. 1 and Pusa Farm 1 oats as well as their differences.

Table XIII.

Yield of oats at the Pusa Farm, 1929-30.

Yields in lb. from ½ acre plots		Difference in lb. (d)	Square of difference (d^2)
B. S. 1	P. F. 1		
1275	1072	+203	
1170	1128	+42	
1187	1148	+39	
1214	1201	+13	
1314	1275	+39	
1326	1326	0	
1298	1371	−73	
1382	1312	+70	
1396	1404	−8	
1363	1187	+176	
1212	1191	+21	
1285	1263	+22	
1203	1312	−109	
1289	1181	+108	
1252	1205	+47	
1168	1232	−64	
1113	1082	+33	
1146	1189	−43	
1152	1292	−140	
	Total	Σ +376	Σ140766

Mean difference $= \frac{376}{19} = 19{\cdot}79$ lb.

Standard deviation $= \sqrt{\frac{140766}{19} - \left(\frac{376}{19}\right)^2} = 83{\cdot}76$

Therefore Student's $z = \frac{19{\cdot}79}{83{\cdot}76} = 0{\cdot}236$

Odds about 5 : 1 in favour of B. S. 1 which are not statistically significant.

This experiment furnishes reliable information regarding the futility of taking very big experimental plots (here half an acre each) in order to estimate the comparative yielding powers of two or more varieties of a cereal crop such as oats. The wide variations in fertility present in a large field are then liable to counterbalance and mask the differences that may be present in the yielding power of the varieties that are to be compared and hence plots of this dimension are absolutely useless for yield trial purposes with cereals. About one-fortieth of an acre is considered to be the correct size for conducting yield trials with cereals. This therefore explains the inconclusive results obtained from the experiment in the larger area as compared with a more comprehensive trial conducted on a smaller scale.

The superiority of the B. S. types for a number of years over the Pusa Farm type led the Imperial Agriculturist to reject the latter and to adopt the former as the standard oat on the Pusa Farm. The B. S. oats are now being distributed on a large scale and the demand for B. S. 1 and B. S. 2 oats is steadily increasing every year ; favourable reports on the good quality and yielding power of these oats are being received continuously from those who have tried them.

These results, therefore, fully bring out the economic value of these two selections of oats and leave no doubt that they will achieve a definite position in agriculture in Bihar and other parts of India.

Summary.

Indian oats belong to the species *Avena sterilis* L. var. *culta*. (synonymous to the American *Avena byzantina* Koch.) and not to the species *Avena sativa* L. as has been reported wrongly in all the Indian floras.

In India the oats are generally sown in October or November and reaped in March or April. The crop grows best on well-drained friable soils of a fair depth and does not suit very light sandy or dense clay soils. It requires the same kind of soil and cultural conditions as wheat or barley. The seed rate generally varies from 70 to 100 lb. per acre and the average yield ranges from about 1,000 to 2,500 lb. per acre depending upon the type of oat and the environmental conditions.

It has been possible to evolve two high yielding and drought-resistant types of oats, *viz.*, B. S. 1 and B. S. 2 from the ordinary mixed Bihar oats. They are both early maturing and fairly disease-resistant, and have proved of considerable value for grain and straw purposes.

A trial of a large number of European and American oats at Pusa has shown the futility of attempting to acclimatize these types. They are invariably all much too late for Indian climate and can hardly be expected to produce normal

yields of grain. Most of them, however, produce excellent and profuse green fodder and are ideal oats for this purpose. The setting of seed in these oats having proved itself to be the limiting factor in their growth in India, hybridization has been resorted to and a number of very promising hybrids combining the early maturity and drought-resistance of B. S. oats and the good grain and profuse-straw producing qualities of the exotics have been secured. These will be classified and described later.

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STUDIES IN INDIAN OATS

II. INHERITANCE OF SOME CHARACTERS IN INTERSPECIFIC CROSSES BETWEEN *AVENA SATIVA* L. AND *AVENA STERILIS* L. VAR. *CULTA*.

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(With Plates LXI-LXIV and six text-figures).

I. INTRODUCTION.

The isolation of two strains of oats, *viz.*, B. S. 1 and B. S. 2, by pure line selection, at the Botanical Section, Pusa, has provided high yielding and draught-resistant types [Shaw and Bose, 1933]. These are early maturing types but their straw in common with that of all Indian oats, is weak and hence these oats are rather susceptible to lodging. The seed colour and quality also of these oats cannot favourably be compared with those of some exotic oats, such as Scotch Potato or Abundance. Most of the exotics on the other hand require a longer growing period and are therefore not amenable to acclimatization in the short cold weather growing season which is general in the plains of North India.

In order to combine the plump grain and profuse straw-producing qualities of some of the exotic oats with the earliness, drought-resistance and growing qualities of the Pusa types, a number of crosses have been effected. This work was started in 1924 and the purpose of the following pages is to present the results obtained in a study of the inheritance of some characters observed in the following crosses :—

Cross I.—Scotch Potato × B. S. 4.

Cross II.—Scotch Potato × B. S. 2.

Cross III.—Abundance × B. S. 4.

It may be pointed out that both the Scotch Potato and the Abundance oats belong to the species *Avena sativa* L. while B. S. 2 and B. S. 4 belong to the species *Avena sterilis* L. var. *culta* which is synonymous with the American *Avena byzantina* Koch. These crosses, therefore, are species crosses, and although some sterile hybrids were obtained as a result of these matings, many viable forms have also been secured. A number of species crosses have been made of late in Europe and America and various desirable combinations and recombinations have been secured. The material obtained as a result of the present work

has yielded some promising hybrids, some of which are now emerging out of the yield trial stage and are being released for distribution.

II. Material and method.

B. S. 2 and B. S. 4 oats are two early-maturing selections from Bihar oats and have gained popularity with the growers. Samples of Scotch Potato and Abundance oats were imported from Carters in England and are both rather late in maturity. These two exotic oats invariably head-out late in the season at a time when almost all the Indian oats are ready for harvest. The great divergence in the time of flowering amongst the Indian and these exotic oats, therefore, was the first difficulty in hybridization work. This difficulty was overcome to a certain extent by sowing the exotic oats three to four weeks before, and the Pusa oats three or four weeks after, the usual date of seeding. In this way it was possible to induce all these types to flower more or less simultaneously.

In order to give them a longer growth period, experiments were conducted in sowing the foreign oats in the month of July and August in earthen pots kept in ice in order to maintain a soil temperature which is usually found in the winter months of India when this crop is grown in this country. This treatment, however, did not seem to influence the periodicity of the heading-out stage to any appreciable extent. These early sown oats headed out only a few days before the normal period.

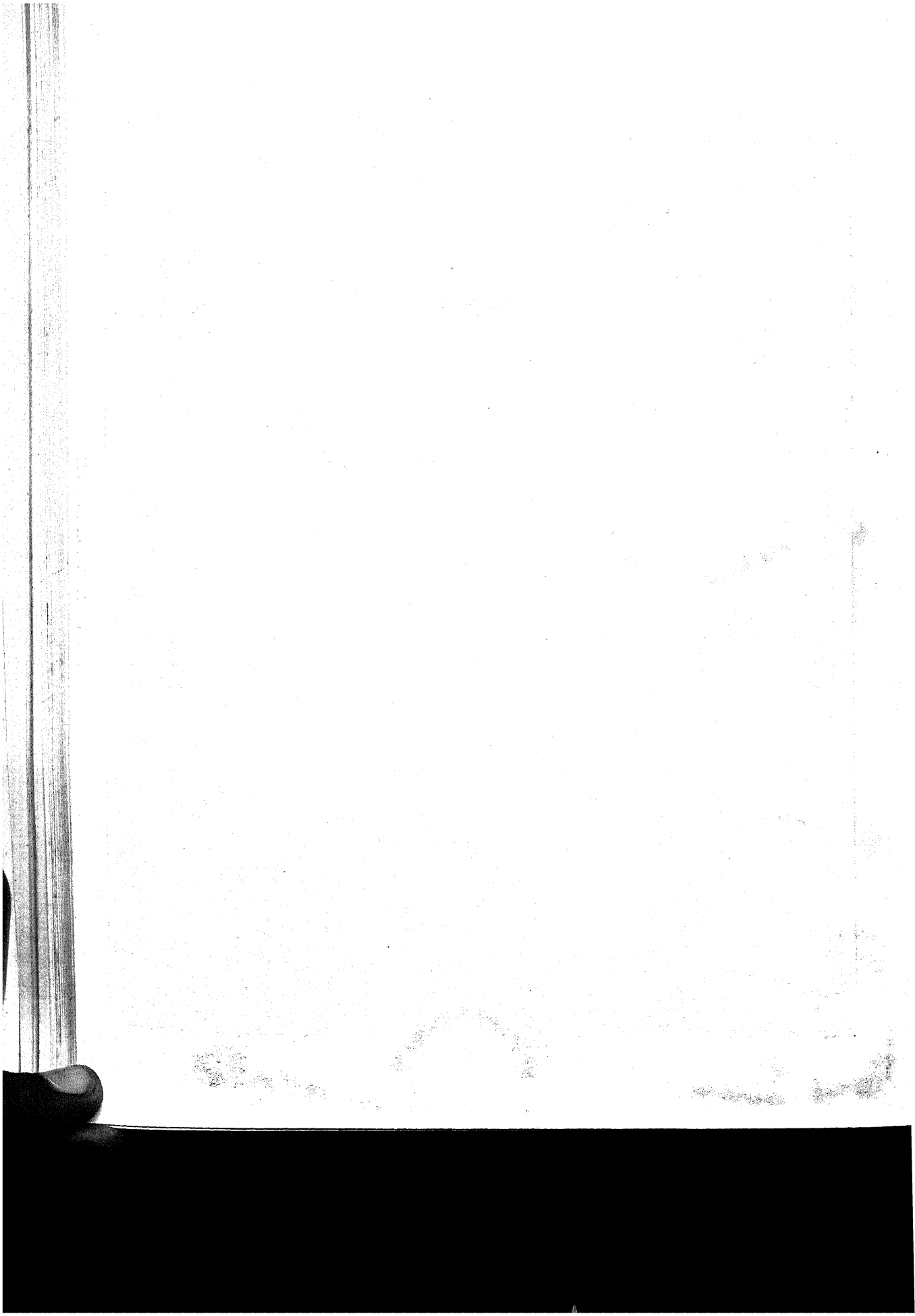
All plants including the parents, F_1, F_2 and F_3 were grown in the field under practically similar conditions. The seed was dibbled by hand in rows three feet apart and the plants were spaced one foot apart in the row.

III. Technique of crossing.

In making crosses, young panicles just emerging out from the leaf-sheaths were selected and all except the topmost 10 or 12 spikelets were cut off from each panicle. The oat spikelet usually consists of two fertile and one abortive florets. The upper florets of the spikelets to be crossed were also removed so that only the lower or primary florets were reserved for further operation. In addition to furnishing uniform material for crossing, this process provided an additional check for detecting crossed and self-fertilized spikelets at the time of harvest, the former possessing only one seed.

The palea and the lemma were separated apart by holding the floret between the thumb and the forefinger and pulling down and holding the palea with the thumb as shown in Plate LXI. The three immature anthers were then removed by means of a fine pair of forceps and care was taken that these forceps were always sterilized with rectified spirit before each operation. The palea were then placed back in position and the whole panicle was covered with either a small

EMASCULATION IN OATS.



muslin bag or by perforated manilla bags specially made for cross-fertilization work. Forty-eight hours after emasculation, mature anthers were brought from the pollen parent plant, placed in well-sterilized Petri dishes and were exposed to the sun for some time. When the anthers began to dehisce, usually between 11 and 12 in the morning, one or more of these anthers were placed within each emasculated floret with a gentle shake of the hand. Sometimes only a little pollen was dusted over the feather-like stigma with the help of a camel-hair brush. After each flower had been treated in this way, the entire panicle was covered with a manilla cross-fertilization bag to prevent the ingress of undesired pollen. Each head was tagged and properly labelled. Fig. 1 depicts the floral structure of oats.

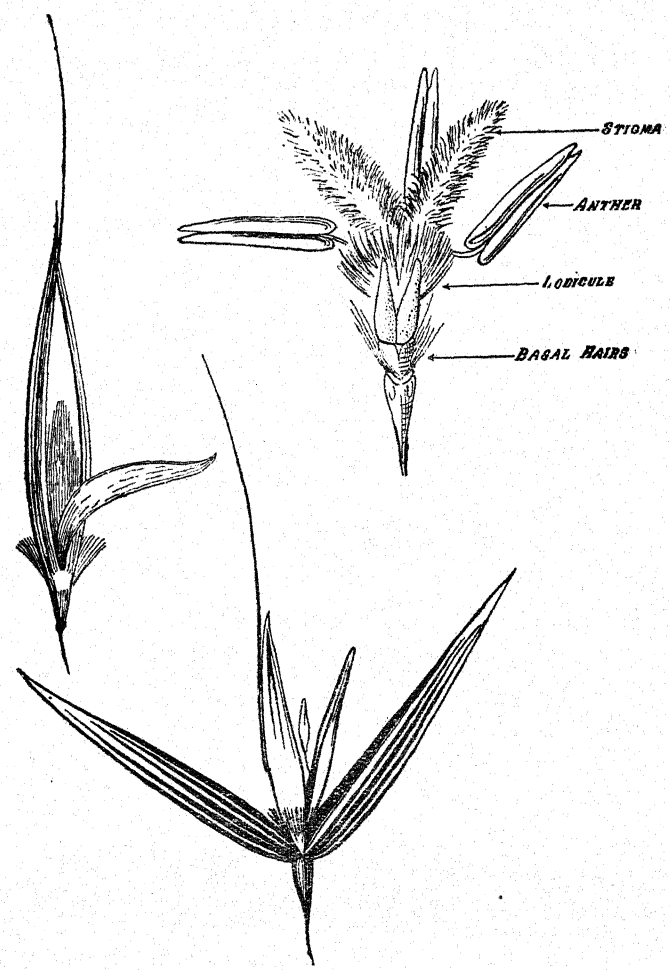


Fig. 1.—Floral structure of oats.

The fact that only one seed can be obtained from each successful operation, acts as a setback in the study of the F_1 generation in oats. This, however, is the general rule in all small-grain breeding and hence it is impossible to raise a large F_1 population.

Although reciprocal crosses were attempted in all cases, success was met with only by taking the exotic variety as the pistillate parent. This is presumably due to factors such as temperature, humidity, the receptive condition of the stigma, especially at this late part of the plant's life, as well as to the pollen tube growth and the activating stimulus of the male nuclei.

Kihara and Nishiyama [1932] have recently published the results of a detailed investigation on the different compatibility of reciprocal crosses of *Avena* in which they report that inter-specific crosses succeed easily between any two *Avena* species having the same chromosome number. These authors assume that the different development of hybrid seeds in reciprocal crosses may be caused by the different strengths of the activating stimulus of the male nuclei on egg and polar nuclei.

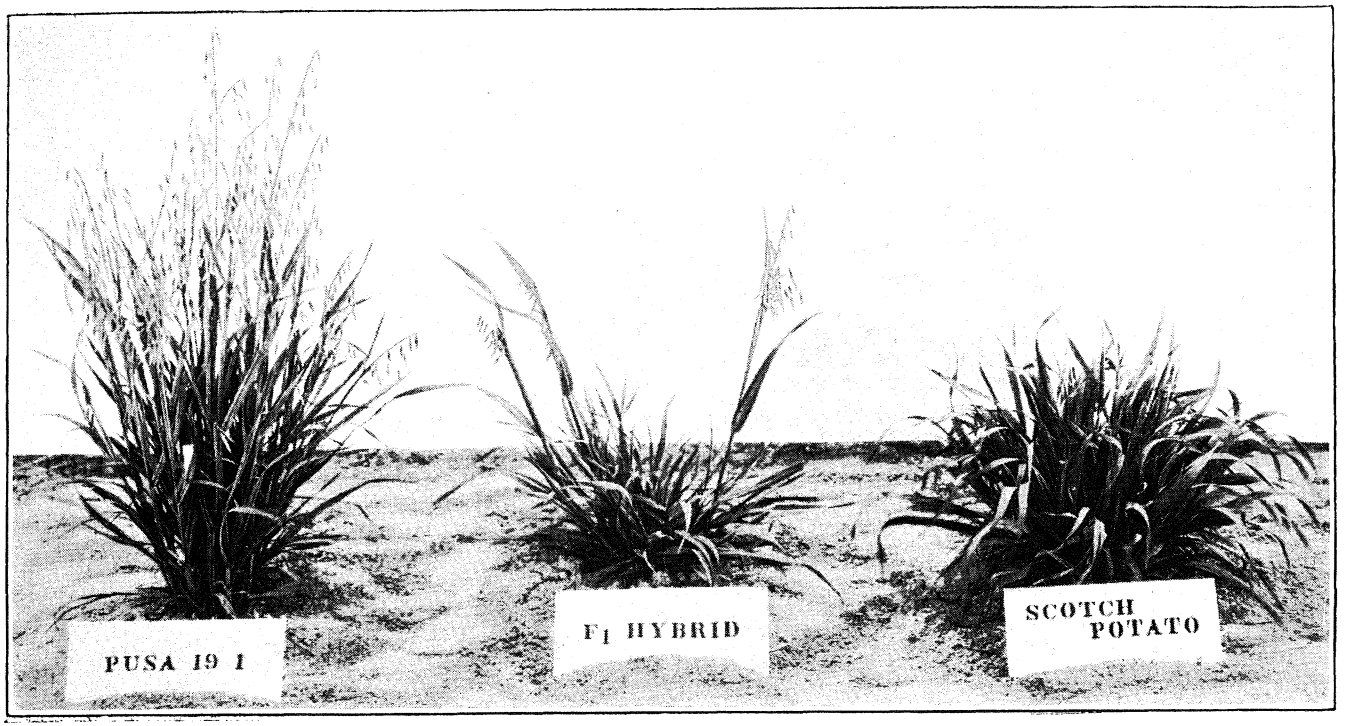
IV. Differentiating characters.

Table I shows a comparison of the main characters studied in the first two crosses.

Table I.

Differentiating characters in Crosses I and II (1927-28).

Characters	B. S. 4 parent	F_1 hybrid Cross I	Scotch Potato parent	F_1 hybrid Cross II	B. S. 2 parent
1. Base	Sterilis	Intermediate	Sativa	Intermediate	Sterilis.
2. Awns	Thin (weak)	Medium (strong).	Thick (strong).	Medium (strong).	Thin (weak).
3. Basal pubescence or hairs	Many	Many—1	Very few	Many—1	Many.
4. Marginal leaf-hairs	Very few	Many	Numerous	Many	Very few.
5. Maturity (*i.e.*, No. of days taken to head out in 1927-28 from the date of sowing).	57 days	72 days	94 days	73 days	65 days.
6. No. of spikelets per panicle	75	116	85	120	80
7. Height of plants in cm.	90	90	96	88	85



PARENTS AND F_1 HYBRID IN CROSS II.

It may be noted that F_1 plants in both the crosses exhibited similar characters and were intermediate for most of them. The number of spikelets per panicle, however, showed a decided increase over the parental forms.

Table II likewise shows the chief characters in Cross III :—

TABLE II.

Differentiating characters in Cross III (1928-29).

Characters	Abundance parent	F_1 hybrid Cross III	B. S. 4 parent
1. Base	Sativa	Intermediate	Sterilis.
2. Awns	Thick (strong)	Medium (strong)	Thin (weak).
3. Basal pubescence or hairs	Very few	Many-1	Many.
4. Marginal leaf-hairs	Almost *nil*	Few	Very few.
5. Maturity (No. of days taken to head out in 1928-29 from the date of sowing).	122 days	72 days	76 days.
6. No. of spikelets per panicle	140	98	75
7. Height of plants in cm.	149	148	128

Here again the intermediate nature of the F_1 hybrid plants is apparent for the majority of characters. The number of spikelets per panicle has also tended to be intermediate between the parental forms, unlike that shown in the previous two crosses.

V. F_1 GENERATION.

Only a single F_1 hybrid plant could be secured in each of the first two crosses and two plants in Cross III. All of them displayed great hybrid vigour and were very healthy. Photographs (Plates LXII and LXIII) show these plants in Crosses II and III wherein remarkable hybrid vigour expressed as yield, growth and increased number of spikelets per panicle and tillers per plant may be well seen. In studies of heterosis, Coffman [1933], obtained variable results from different crosses of oats. In crosses of Richland × Fulghum and Richland × Markton, the F_1 plants in the first cross were taller, bore more culms, weighed

more and yielded more grain than the larger parent, while the F_1 plants in the second cross displayed only an increased grain yield and grain/straw ratio. The fact that in some F_1 hybrids a number of parts of the plant may show increased size does not necessarily imply genetic linkage between the different size factors. In cases in which heterosis is definitely restricted to a single part in the F_1 plant the absence of such linkage between size factors is definitely indicated.

Basal articulation is of the *sativa*-type in the Scotch Potato and Abundance oats, while it is *sterilis*-like in the B. S. parents. The F_1 hybrids show an intermediate condition in that the base is somewhat of the *sterilis* nature but the "sucker mouth" scar typical of this species is not at all prominent.

The awns in both the exotic oats are thick or strong and in the B. S. oats they are invariably thin or weak. The F_1 plants, however, have awns of medium thickness. At first it was found rather difficult to differentiate between the thick and medium awns but later it was observed that in the crosses under study the thick basal portion of the true strong awn generally extended beyond the length of the glumes. The medium strong awns on the other hand generally showed their basal thickness to fall short of the length of the flowering glumes.

The basal hairs in the European oats were "very few" and those in the B. S. oats were "many". The F_1 plants also had many hairs but these were always less than those found in the B. S. parents. This phenotype was therefore called "Many-1" and was seen to behave as the double dominant in the F_2.

There was also a remarkable increase in the number of spikelets per panicle in the F_1 plants in Crosses I and II.

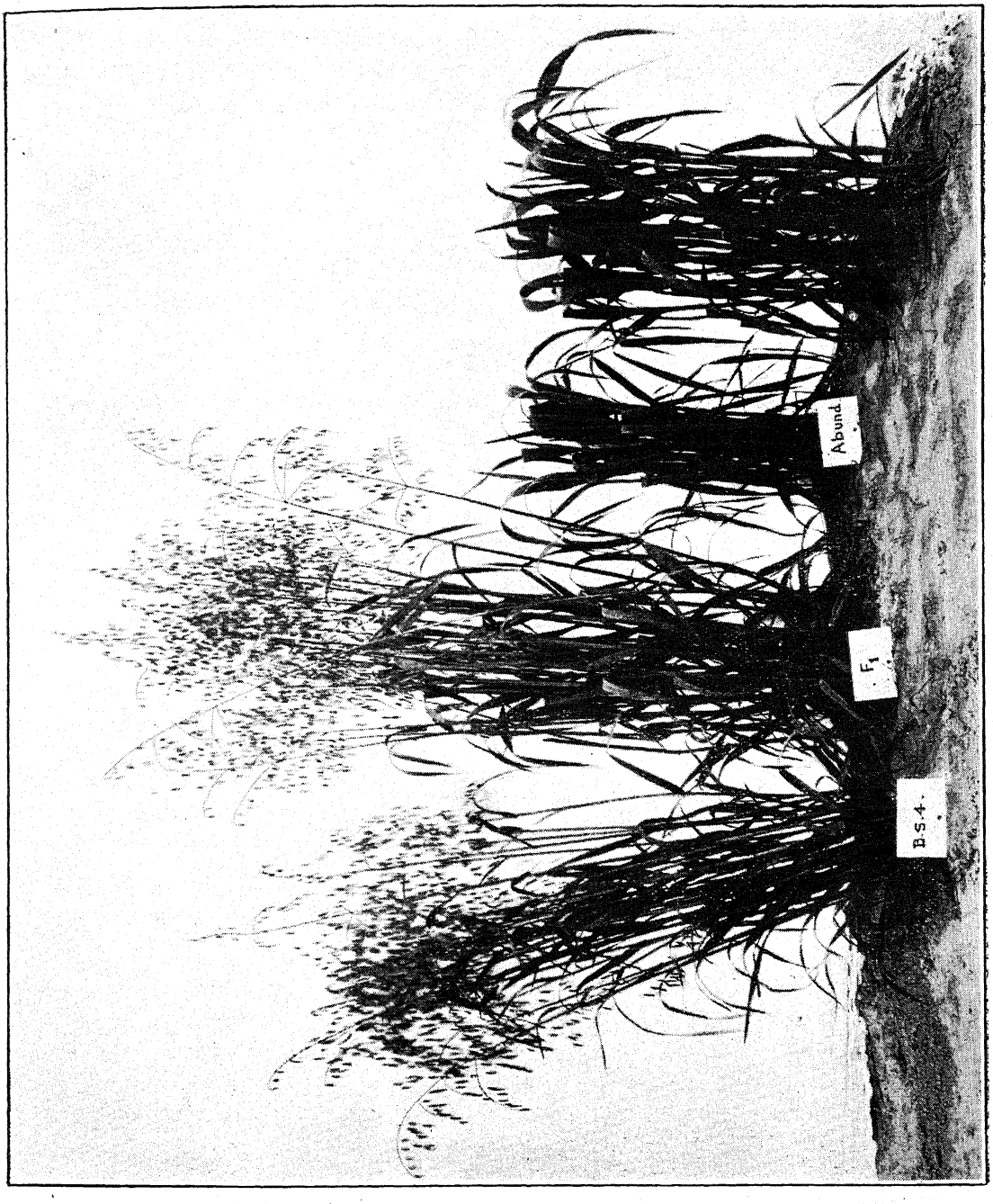
VI. F_2 AND F_3 GENERATIONS.

Seeds obtained from selfed F_1 plants in the crosses under discussion were dibbled by hand under field conditions in rows 3 feet apart and were spaced one foot apart in the row. Plants were carefully thinned out later and only one seedling was allowed to occupy each space so as to avoid errors in diagnosis. The plants were mostly very vigorous owing to :—

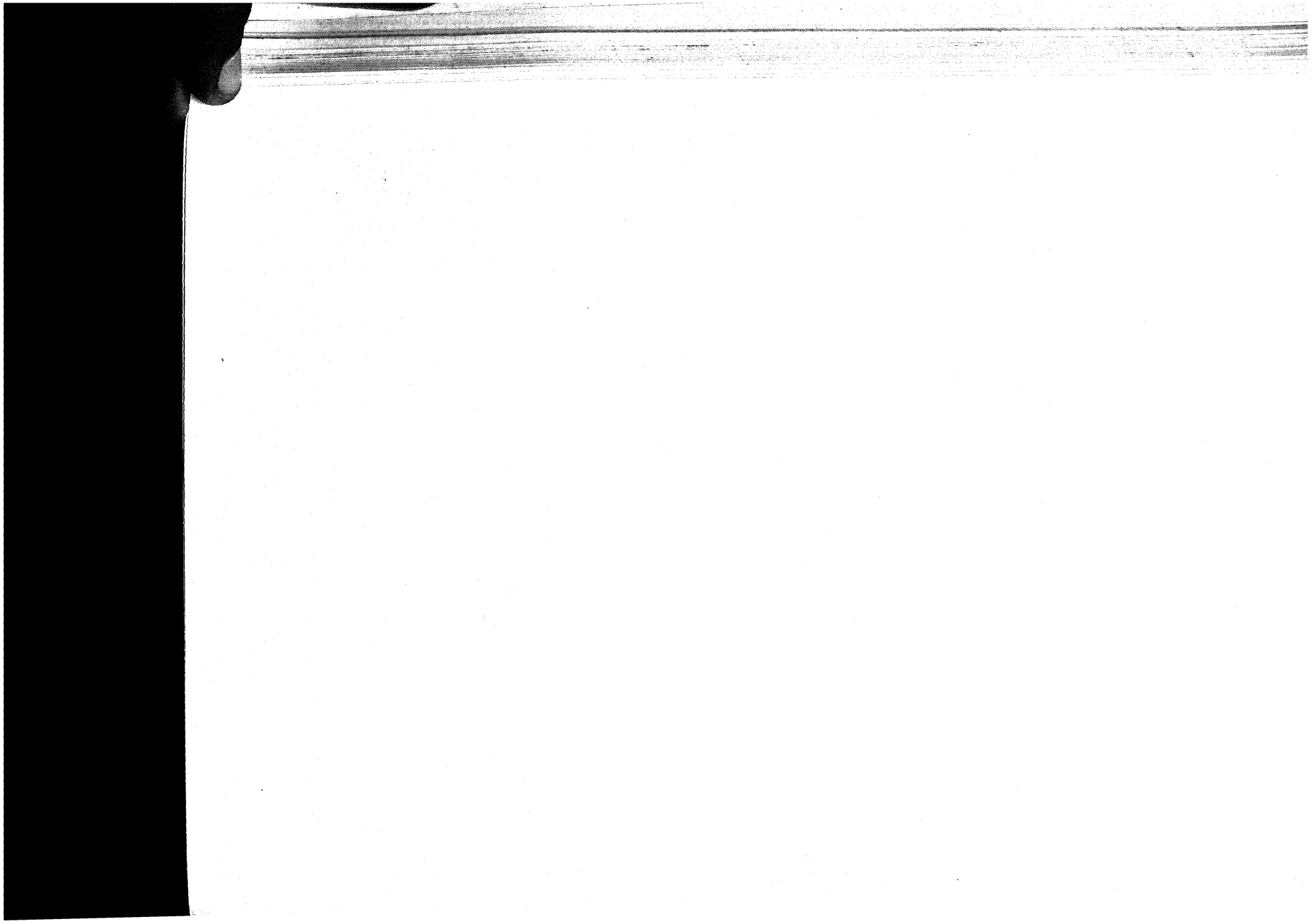
(*i*) hybrid vigour and

(*ii*) the spacing given to each.

A number of plants in each cross were extremely late and did not flower at all. These were presumably responsible to a great extent for the discrepancies which occurred between observed and expected frequencies in the study of inheritance of characters in the spikelet and in the inheritance of time of maturity.



PARENTS AND F_1 HYBRID IN CROSS III.



The following characters were studied in detail and their genetic behaviour will be considered in the following pages :—

1. Qualitative characters.

1. Basal articulation.
2. Nature of awns.
3. Basal pubescence.
4. Hairs on the margin of the leaf.

2. Quantitative characters.

1. Maturity as measured by the number of days taken to head-out.
2. Average number of spikelets per panicle.
3. Height of plants.

VII. Spikelet.

As mentioned in the previous pages all the three crosses under consideration were species crosses between *A. sativa* L. and *A. sterilis, culta* L. Lately Coffman, Parker and Quisenberry [1926] have adopted the use of the name *A. byzantina* Koch. for the cultivated varieties of *A. sterilis, culta* L., and this is the terminology favoured by many workers. The term *sterilis* used in the subsequent pages should, therefore, be taken to indicate this cultivated variety and not the true wild *sterilis* species from which all cultivated forms of this species have presumably descended. The greatest stress in these studies was given to diagnosing the spikelet character in the F_2, F_3 and subsequent generations, as it is only here that the two species can be easily separated. Three important characters were involved in this :—

(*a*) Basal articulation,
(*b*) Nature of awns,
and (*c*) Basal pubescence.

In the present studies the population has been smaller than is usual in such work owing to the fact that only one single plant in each cross could be obtained in the F_1 generation, but the nature of the inheritance of the important characters studied is fairly evident.

(1) Basal articulation.

In the *sterilis* type the lower grain has marked articulation with the main axis and is readily detached leaving a cup-like or 'sucker-mouth' scar, slightly to one side and running obliquely to the length of the grain. This character is responsible for the shattering of Indian oats which sometimes occurs if the crop is left in the field till it is dead ripe. The upper grain has no articulation and when detached from the rest of the spikelet it carries away from the lower grain the greater part

of the rachilla which breaks leaving only a stump with a jagged edge to the lower grain (Fig. 2).

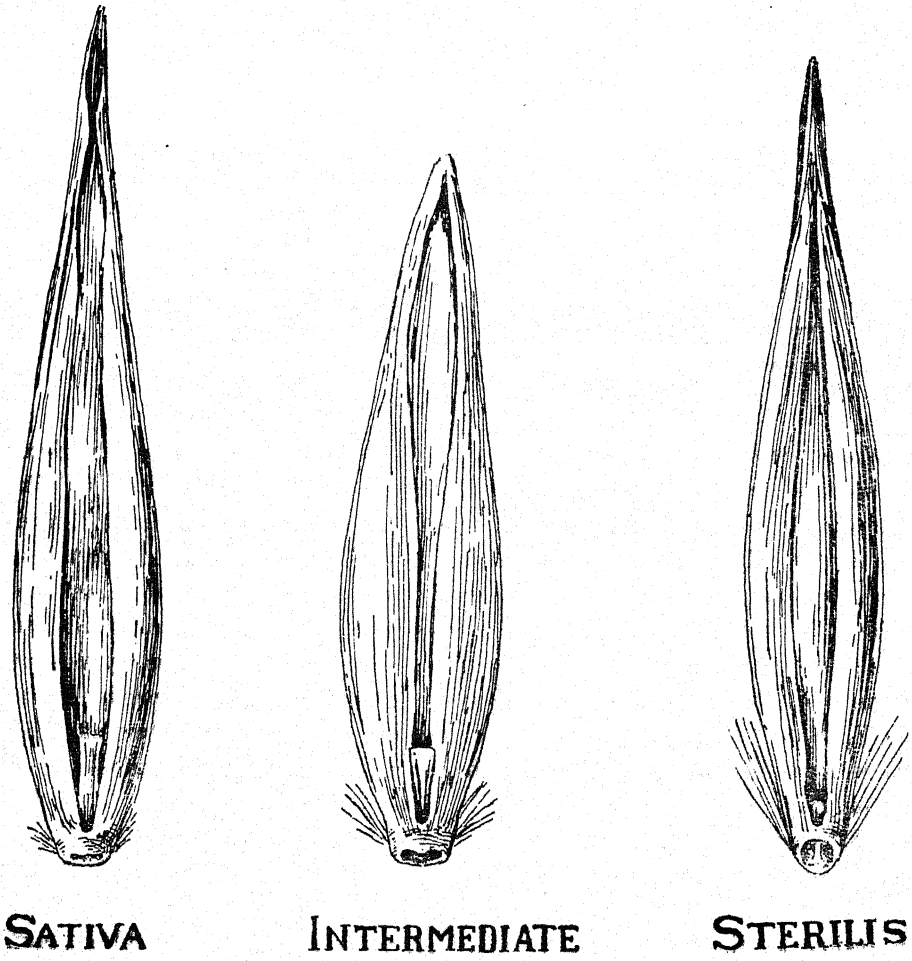


Fig. 2.—Type of base in *A. sativa*, F_1 (intermediate), and *A. sterilis*.

In the *sativa*-type, however, the base of the lower grain is confluent with the peduncle and is separated therefrom by a distinct fracture. The upper grain in this case, on the other hand, is articulated and is readily detached from the rachilla which remains attached to the lower grain.

The intermediate condition, as represented by the spikelets in the F_1 plants and the heterozygotes in F_2 and F_3, generally has a somewhat *sterilis*-like lower floret, but the sucker-mouth is not at all prominent. The upper floret here sometimes behaves more or less like the *sativa*-type. Consequently this type of base is not always clearly recognized.

Love and Craig [1918] found a single factor difference for the presence and absence of basal articulation. Surface [1916] obtained a 1 : 2 : 1 ratio for this character in a cross between *A. fatua* and *A. sativa*, var. Kherson. Fraser [1919] found a 1 articulate base to 3 intermediate and *sativa* type in a cross between Burt (*A. sterilis*) × 'Sixty day' (*A. sativa*) oats. Tschermak [1929] records a unit factor difference in type of floret separation in crosses between *A. fatua* × *A. sterilis*, the *fatua* base being recessive. Florell [1931] in a recent paper also reports that the type of floret separation is governed by a unit factor in 6 interspecific oat crosses but found a 2-factor difference on a 15 : 1 non-articulate to articulated type of base in one cross.

Our present observations given below also show a unit factor difference in the type of floret separation in three crosses between *A. sativa* × *A. sterilis*, the *sterilis* type of base always being recessive.

The F_1 plants invariably had an intermediate type of base.

In the F_2 the following phenotypes and frequencies occurred:—

TABLE III.

Segregation in F_2 for type of base on a 3 : 1 basis.

Cross	Type of base		Total No. of plants	Dev.	P. E.	Dev./P. E.
	Sativa and *intermediate*	*Sterilis*				
I. *Scotch Pot.* × *B. S. 4*—						
Observed	227	80	307	3·25	5·12	0·63
Expected	230·25	76·75				
II. *Scotch Pot.* × *B. S. 2*—						
Observed	200	81	281	11·25	4·89	2·30
Expected	210·75	70·25				
III. *Abun.* × *B. S. 2*—						
Observed	209	72	281	1·75	4·89	0·36
Expected	210·75	70·25				

It may be noted that the ratio of the deviation to the probable error is extremely low in Crosses I and III and almost within limits in Cross II. This suggests clearly that a single factor difference is present between the *sterilis* and the non-*sterilis* (*sativa* and intermediate) types of base. The larger deviation in Cross II is due to the fact that the characters of a number of very late plants which did not flower at all owing to the advent of west winds late in the season, could not be incorporated in the diagnosis.

The 3 : 1 ratio is again confirmed by the F_3 behaviour of a number of selections taken. Thus—

TABLE IV.

F_3 breeding behaviour for inheritance of type of base.

Cross	No. of families and the nature of F_2 parent	F_3 behaviour	No. of families	
			Observed	Expected
I	59. *Sativas* and *intermediate*	Pure sativas	17	19·7
		Segregating (3 : 1)	42	39·3
	17. *Sterilis*	Pure sterilis	17	17
II	53. *Sativas* and *intermediate*	Pure sativas	20	17·7
		Segregating (3 : 1)	33	35·3
	13. *Sterilis*	Pure sterilis	13	13
III	33. *Sativas* and *intermediate*	Pure sativas	5	11
		Segregating (3 : 1)	28	22
	13. *Sterilis*	Pure sterilis	13	13

The *sterilis* selections have invariably always bred true and therefore show that they form the recessive phenotype. In the segregating families, 6 families each were studied in detail in Crosses I and II respectively and all the families were studied in Cross III for this character. The following total frequencies were observed :—

TABLE V.

Segregation in F_3 for type of base in some selections.

Cross	No. of families studied	Frequency	Type of base		Total No. of plants	Dev.	P. E.	$\frac{\text{Dev.}}{\text{P. E.}}$
			Sativa and *intermediate*	*Sterilis*				
I	6	Observed	413	133				
		Expected	409·5	136·5	546	3·5	6·82	0·51
II	6	Observed	408	161				
		Expected	426·75	142·25	569	18·75	6·97	2·69
III	28	Observed	1348	434				
		Expected	1336·5	445·5	1782	11·5	12·33	0·93

These figures definitely prove that a single factor difference is present between the *sterilis* and the non-*sterilis* types of base in the three crosses under study. This is therefore in conformity with the observations of other workers.

(2) *Nature of awns.*

The awn is sometimes described as an aerial prolongation of the middle vein of the dorsal palea emerging therefrom between the apex and the base. In form it is cylindrical or somewhat flattened in its lower portion. In the Scotch Potato and the Abundance oats the awn is present on the primary floret only and has a thick brownish black basal portion, while both the Pusa parents, *viz.*, B. S. 2 and B. S. 4 have thin awns always on the primary and occasionally on the upper floret also.

Oat breeders elsewhere have studied the inheritance of the awn character from the point of view of the degree of awning rather than of the nature of awning. In the present case the nature of awning alone has been studied.

Different varieties of oats may show all possible degrees of awning, from strong awns on both florets to a completely awnless condition. Nilsson-Ehle [1908] obtained a transgressive segregation for awns. Love and Fraser [1917] working with several varieties report a monofactorial mode of inheritance of the strong " or weak " awned type to the completely awnless one. These results were confirmed by Fraser [1919] in a cross between Burt and 'Sixty-day' oats. Zinn and Surface [1917] crossing a *sativa-nuda* with Victory observed a 3 : 1 ratio between plants with medium strong to strong awns and plants with weak awns. Coffman, Parker and Quisenberry [1925] found that probably several factors were involved in the inheritance of the awns of the Burt oat and that twisted awn had a different genetic behaviour from the other types. Lunden [1925] found one factor responsible for the inheritance of geniculate awns.

The exotic oats, Scotch Potato and the Abundance are characterised by the presence of 'thick' or 'strong awns.' B. S. 2 and B. S. 4 on the other hand have 'thin' or 'weak' awns. The F_1 progenies all had 'strong' awns of medium development. It was observed that the basal portion of the true 'thick' awns had a dark thickened region extending beyond the length of the flowering glume, whereas in the 'medium' awn this thickening went only up to about three quarters the length of the flowering glume. It was not always possible, therefore, to distinguish between these two phenotypes and hence these two classes have been combined to form the 'strong' awn class in contrast to the distinct thin or 'weak' awned class.

The F_2 showed the following frequencies :—

TABLE VI.

Segregation in F_2 for type of awn on a 3 : 1 ratio.

Cross	Frequency	Type of awn		Total No. of plants	Dev.	P. E.	Dev. / P. E.
		Strong	Weak				
I	Observed	216	91				
	Expected	229·5	76·5	307	14·5	5·12	2·83
II	Observed	212	69				
	Expected	210·75	70·25	281	0·75	4·89	0·15
III	Observed	205	76				
	Expected	210·75	70·25	281	5·75	4·89	1·18

The fit between observed and expected frequencies is close and indicates a clear monohybrid ratio.

This is confirmed by the behaviour of a number of F_3 progenies studied in the following year :—

TABLE VII.

F_3 behaviour for inheritance of type of awn.

Cross	No. of families and the nature of F_2 parent	F_3 behaviour	No. of families	
			Observed	Expected
I	61. Strong	Pure for strong	19	20·3
		Segregating (3 : 1)	42	40·6
	15. Weak	Pure for weak	15	15
II	53. Strong	Pure for strong	20	17·7
		Segregating (3 : 1)	33	35·3
	13. Weak	Pure for weak	13	13
III	33. Strong	Pure for strong	5	11
		Segregating (3 : 1)	28	22
	13. Weak	Pure for weak	13	13

Excepting for rather wide divergence in the pure and segregating families of the 'strong' phenotype in Cross III, the F_3 behaviour in all the crosses confirms the F_2 hypothesis of the monogenic nature of segregation for this character. In Cross III fewer homozygous 'strongs' were perhaps selected by chance.

A few segregating families studied in detail in the F_3 gave the following phenotypes and total frequencies:—

TABLE VIII.

Segregation in some F_3 selections for type of awns.

Cross	No. of families studied	Frequency	Type of awn		Total No. of plants	Dev.	P. E.	Dev./P. E.
			Strong	Weak				
I	8	Observed	562	190				
		Expected	564	188	752	2·00	8·01	0·25
II	8	Observed	464	145				
		Expected	456·75	152·25	609	7·25	7·21	1·00
III	28	Observed	1330	452				
		Expected	1336·5	445·5	1782	6·50	12·33	0·53

Relation of base and awn.—While studying the basal articulation and the awn characters it was observed that the *sterilis* base mostly had a 'weak' awn indicating linkage between these two characters. Taking these characters together we have :—

TABLE IX.

Relation of base and awn in F_2.

Cross	Frequency	*Sativa*		*Sterilis*		Total No. of plants	Cross-over value
		Strong	Weak	Strong	Weak		
I	Observed	195	28	21	62	306	Per cent. 17·0
	Expected (9 : 3 : 3 : 1)	172·13	57·37	57·37	19·13		
II	Observed	173	27	33	48	281	23·0
	Expected (9 : 3 : 3 : 1)	158·0	52·7	52·7	17·6		
III	Observed	202	7	3	69	281	5·3
	Expected (9 : 3 : 3 : 1)	158·0	52·7	52·7	17·6		

The frequencies in the parental classes are greatly beyond expectation, while taken singly each character shows a good 3 : 1 fit. Linkage is therefore indicated and the linkage value in each case is shown in the last column of the above table.

The high cross-over values in Crosses I and II, *viz.*, 17·0 and 23·0 per cent. respectively, suggest that a number of pure breeding cross-over phenotypes may

occur and indeed this is what has actually happened in F_3. In Cross III where the cross-over value is only 3·3 per cent. no pure breeding cross-overs were realised.

In Cross I all F_3 families which bred true for *sativa* type of base also bred true, for strong awns, but of 17 families which were pure for *sterilis* type of base, 15 families bred true for weak awns and two were homozygous for medium strong awns. Similarly in Cross II it was found that 18 pure breeding *sativa* families bred true for strong awns, while 2 *sativa* families had weak awns, and also in 13 families which were pure for the *sterilis* character, 11 families bred true for weak awns and two families were homozygous for medium strong awns. In Cross III, however, all pure breeding *sativas* were pure for strong awns and all homozygous *sterilis* families possessed weak awns. In other words wherever there was a high cross-over value the chances of securing a greater number of homozygous cross-over phenotypes were also very great. Tabulating this statement we get :—

TABLE X.

Relation of type of base and nature of awns in homozygous F_3 families.

Cross	No. of families			
	Sativa-base		*Sterilis*-base	
	Strong	Weak	Strong	Weak
I	17	0	2	15
II	18	2	2	11
III	5	0	0	13

(3) Basal pubescence.

Cultivated varieties of oats differ in the amount and in the presence and absence of basal hairs on each side of the callus of the spikelet. These hairs also differ in size, some are quite long (5 mm. or more), others are medium long while others again are short and hardly exceed one or two millimetres in length. It has been reported that the inheritance of basal hairs depends on one factor in some crosses and two factors in others [Hays and Garber, 1927].

Fraser [1919] and Love and Craig [1918] have definitely found in their studies a monohybrid ratio for the inheritance of pubescence. Zinn and Surface [1917] also found a monfactorial segregation of long and short hairs at the base of the grain and this was in agreement with the results of Nilsson-Ehle [1909] and those of Coffman, Parker and Quisenberry [1925]. Zinn and Surface obtained a bifactorial segregation showing 15 pubescent to 1 smooth in a cross between *A. sativa* and *A. nuda*.

In our present study a two-factor difference has been found to exist for this character. The exotic parents, *viz.*, Scotch Potato and Abundance oats have a 'very few' hairs at the base of their lower grain whereas B. S. 2 and B. S. 4 have 'many' hairs. The F_1 plants in all the three crosses invariably had 'many' hairs but the hairs were distinctly fewer than those found in the B. S. parents. In the following description such hairs will be termed 'many-1' in contrast to 'many' like the B. S. parents. The F_2 progenies showed plants in which pubescence was not only intensified but also some which lacked pubescence altogether, thus suggesting that at least two factors were responsible for the inheritance of this character.

Some difficulty was experienced during the initial stages of this study in recognizing the difference between the 'many-1' and 'many' classes and hence these two phenotypes were combined in the F_2 studies of the first two crosses. With greater experience the character was fairly well differentiated in Cross III.

The F_2 progenies showed the following segregations :—

TABLE XI.

Segregation in F_2 for basal pubescence.

Cross	Nature of hairs				Total No. of plants	χ^2	P
	Many-1	Many	Very few	*Nil*			
I. Scotch Pot. × *B. S. 4.*							
Observed	..	233	59	15	307		
Expected on 12 : 3 : 1	..	230·4	57·6	19·2			
$(O-C)$	..	2·6	1·4	4·2			
$\frac{(O-C)^2}{C}$	..	0·0293	0·0341	0·9200		0·9834	Between 0·70 and 0·50.
II. Scotch Pot. × *B. S. 2.*							
Observed	..	208	56	17	281		
Expected on 12 : 3 : 1	..	211·2	52·8	17·6			
$(O-C)$	..	3·2	3·2	0·6			
$\frac{(O-C)^2}{C}$	..	0·0485	0·1940	0·0205		0·2630	Between 0·90 and 0·80
III. Abun. × *B. S. 4.*							
Observed	155	50	61	14	281		
Expected 9 : 3 : 3 : 1	158·4	52·8	52·8	17·6			
$(O-C)$	3·4	2·8	8·2	3·6			
$\frac{(O-C)^2}{C}$	0·0730	0·1485	1·273	0·7362		2·2307	Between 0·70 and 0·50

The fit in every case is very good.

The dihybrid theory for F_2 for the inheritance of this character is further confirmed by the observations in F_3. The results in F_3, however, are obscured

slightly by our failure to differentiate between the phenotypes 'many' and 'many-1' when these occurred in the same culture. In other words the segregation of the genotype **CCDd** (page 805) was not clearly distinguished from the pure breeding **CCDD** and hence these two genotypes have been considered together in the following table.

TABLE XII.

F_3 behaviour for the inheritance of basal pubescence in oat crosses.

Cross	No. of families and the nature of F_2 parent	F_3 behaviour	No. of families		No. of families studied in detail
			Observed	Expected	
III	25 many-1	Pure for many-1	8	8·3	8
		Segregating (3 : 1)	5	5·5	5
		,, (Like F_2)	12	11·1	12
	13 many	Pure for many	4	4·3	4
		Segregating (3 : 1)	9	8·6	9
	6 very few	Pure for very few	3	2·0	3
		Segregating (3 : 1)	3	4·0	3
	2 *nil*	Pure for *nil*	2	2·0	2
II	18 many-1	Pure for many-1	8	6·0	8
		Segregating	10	12·0	3 (3 : 1).
					3 (like F_2).
					4 (not studied in detail).
	22 many	Pure for many	9	7·3	9
		Segregating (3 : 1)	13	14·7	3
	23 very few	Pure for very few	9	7·7	9
		Segregating (3 : 1)	14	15·3	2
	3 *nil*	Pure for *nil*	3	3·0	3
I	29 many-1	Pure for many-1	8	9·7	8
		Segregating	21	19·3	3 (3 : 1).
					7 (like F_2).
					11 (not studied in detail).
	24 many	Pure for many	12	8·0	12
		Segregating (3 : 1)	12	16·0	4
	20 very few	Pure for very few	9	6·7	9
		Segregating	11	13·3	..
	3 *nil*	Pure for *nil*	3	3·0	3

It will be noted from the above table that the observed and expected numbers of families agree to a fairly good extent in most cases and thus uphold the theory that at least two factors are responsible for the inheritance of the pubescence character in the three crosses under observation. The somewhat large deviations observed in the pure breeding 'many' and 'very few' classes in Crosses I and II may probably be due to the selections for F_3 having been made rather from an

economic point of view with the object of securing agriculturally superior types than from a genetic standpoint.

Of the segregating families in the F_3, 29 were studied in Cross III, while only 14 were studied in detail in Cross I and 11 in Cross II. These showed the following segregations :—

TABLE XIII.

Segregation in some selections in F_3 for basal pubescence.

Cross	No. of families studied and nature of segregation	Total frequencies	Many-1	Many	Very few	*Nil*	Total number of plants	Dev.	P. E.	Dev./P. E.
III	12 like F_2 9 : 3 : 3 : 1.	Observed	563	189	174	58	984			
		Expected	553·5	184·5	184·5	61·5				
		(*O*—*C*)	9·5	4·5	10·5	3·5				
		χ^2	=1·0697		P=between 0·80 and 0·70					
	5·3 many-1 : 1 very few.	Observed	299	..	109	..				
		Expected	306	..	102	..	408	7·0	5·90	1·19
	9·3 many : 1 *nil*	Observed	..	608	..	227				
		Expected	..	626·25	..	208·75	835	18·25	8·44	2·15
	3·3 very few : 1 *nil.*	Observed	..	..	197	51				
		Expected	..	..	186	62	248	11·0	4·60	2·39
II	3-like F_2 9 : 3 : 3 : 1.	Observed	122	34	35	15				
		Expected	115·92	38·64	38·64	12·88	206			
		(*O*—*C*)	6·08	4·64	3·64	2·12				
		χ^2	=1·5683		P=between 0·70 and 0·50					
	3·3 many-1 : 1 very few.	Observed	195	..	62	..				
		Expected	193	..	64	..	257	2·0	4·68	0·43
	3·3 many : 1 *nil.*	Observed	..	167	..	45				
		Expected	..	159	..	53	212	8·0	4·25	1·88
	2·3 very few : 1 *nil.*	Observed	..	..	137	35				
		Expected	..	..	129	43	172	8·0	3·83	2·09
I	7-like F_2 9 : 3 : 3 : 1.	Observed	342	121	125	43				
		Expected	355·05	118·35	118·35	39·45	631			
		(*O*—*C*)	13·05	2·65	6·65	3·55				
		χ^2	=1·2327		P=between 0·80 and 0·70					
	3·3 many-1 : 1 very few.	Observed	200	..	72	..				
		Expected	204	..	68	..	272	4·0	4·82	0·83
	4·3 many : 1 *nil.*	Observed	..	268	..	101				
		Expected	..	276·75	..	92·25	369	8·75	5·61	1·56

The fit between observed and expected frequencies is sufficiently close when statistically tested to show that the inheritance of basal hairs in the three crosses under consideration depends on at least two factors.

VIII. Size of basal hairs.

As reported previously Zinn and Surface [1917] found a monofactorial segregation of long and short hairs at the base of the grain and this was in agreement with the results obtained by Nilsson-Ehle [1909].

Nine families which were breeding true for 'many' basal hairs, thin awns and *sterilis* base in the F_3 generation of Cross III were examined in detail for the inheritance of the size of hairs. These behaved as follows :—

4 families bred true for short hairs,

3 families bred true for long hairs, and

2 families segregated on a 3 : 1 basis, thus :—

Table XIV.

Segregation in 2 families of F_3 for size of basal hairs.

Cross	No. of families studied	Frequency	Size of hairs		Total Number of plants	Dev.	P. E.	Dev./P. E.
			Long	Short				
III	2	Observed	109	35	144			
		Expected	108	36		1	3·50	0·286

The fit is extremely close indeed and indicates a monohybrid type of segregation for this character which is independent of the gene for the degree of basal pubescence. The data are undoubtedly very meagre but certainly in agreement with the observations of other workers.

IX. Leaf-hairs.

The Scotch Potato oats have numerous hairs on the margin of their leaves while the B. S. types 2 and 4 both have only a very few hairs restricted mainly to the lower one-third of the leaves. The F_1 plants in both Crosses I and II had a medium amount of hairs, more in extent than the B. S. parents but certainly much less than the Scotch Potato parent. These will be called 'many' hairs hereafter. The nature of leaf-hairs may be gauged from Fig. 3.

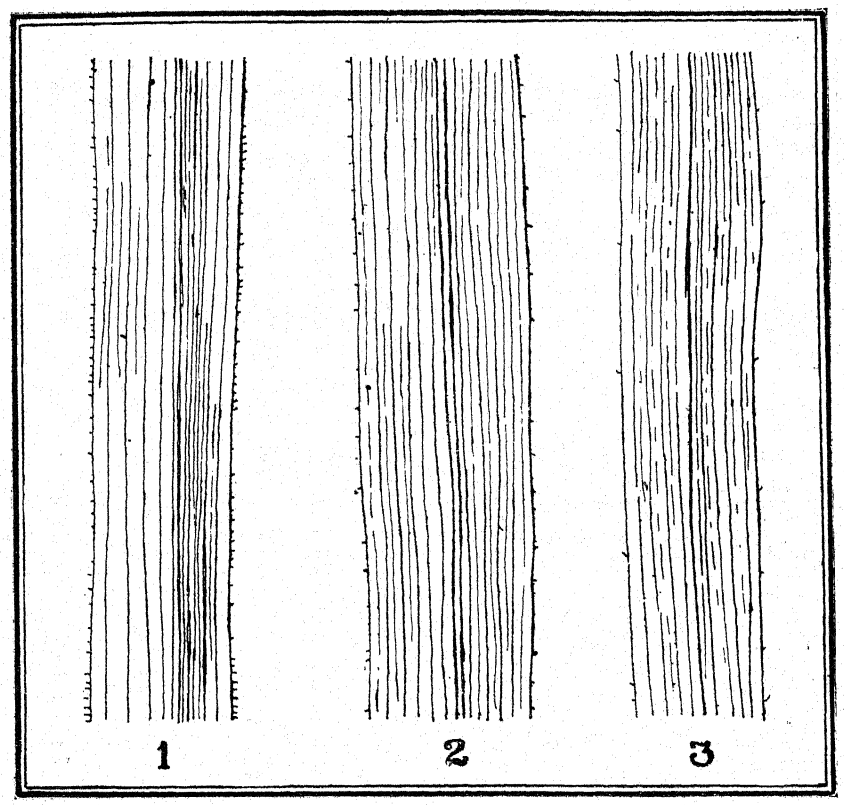


Fig. 3.—Marginal leaf-hairs in oats.

1. Numerous leaf-hairs in Scotch Potato;

2. Many leaf-hairs in F_1 hybrid; and

3. Few leaf-hairs in B. S. 2 oats.

The inheritance of leaf-hairs was studied in detail in Cross II. No record about the inheritance of this character by other workers is known to us. Two

factors appear to be responsible for the inheritance of leaf-hairs. The F_2 showed the following phenotypes and frequencies :—

TABLE XV.

Segregation of leaf-hairs in F_2 on 9 : 3 : 4 basis.

Cross	Year	Frequency	Nature of leaf-hairs			Total number of plants	χ^2	P
			Many like F_1	Numerous like S. Pot.	Few like B. S. 2			
II	1927-28	Observed	108	72	60	240		
		Expected	135	45	60			
		(O—C)	27	27	0		21·6	Less than 0·01.
II	1929-30	Observed	70	28	33	131		
		Expected	73·8	24·5	32·7			
		(O—C)	3·8	3·5	0·3		0·6993	Between 0·80 and 0·70.

It is evident from the above table that the 'many' like F_1 phenotype was easily recognized in 1929-30 and hence fits in this class were almost perfect but some difficulty was experienced in the first year, 1927-28, in differentiating between the 'many' and 'numerous' and therefore the fit between observed and expected frequencies in these phenotypes was badly out although the frequencies in the 'few' phenotype were quite close. A two-factor difference is, however, evident in both years and is finally confirmed by the behaviour of the F_3 generation.

Twenty-nine families studied in the F_3 for this character showed that

10 families were homozygous for 'many' leaf-hairs,

7 families were homozygous for 'numerous' leaf-hairs,

3 families were homozygous for 'few' leaf-hairs, and

9 families segregated as follows :—

TABLE XVI.

Segregation in F_3 of marginal leaf-hairs.

Cross	Nature of parent	No. of families studied	Frequency	Nature of leaf-hairs			Total number of plants	Dev.	P. E.	Dev./P. E.
				Many	Numerous	Few				
II	Many	2	Observed	116	33	47	..	..	..	..
			Expected on 9 : 3 : 4 (Like F_2)	110·25	36·75	49	196	..	..	..
			(O—C)	5·75	3·75	2	..	..	..	..
			χ^2 =	0·7695	P = between 0·70 and 0·50					
		2	Observed	155	37	..	..	..	..	..
			Expected on 3 many : 1 numerous	144	48	..	192	11	4·05	2·72
		5	Observed	337	..	116	..	..	..	..
			Expected on 3 many : 1 few	339·75	..	113·25	453	2·75	6·21	0·44

It may be observed that the 'numerous' class in one case was again deficient but this is perhaps due to mistaken diagnosis in having included some plants having this character with those having 'many' leaf-hairs.

This and other observations with some other crosses to be reported later, therefore, confirms that the inheritance of this character depends on the inter-action of at least two factors.

X. Inheritance of quantitative characters.

A number of quantitative characters have been studied in the present crosses and the more important ones will be discussed here. The influence of the environment on such quantitative characters as the number of tillers per plant or the length and breadth of leaves of oats or other cereals sometimes greatly obscures the true mode of inheritance of such characters. But given approximately the same conditions for plants of all generations, parents, F_1, F_2, and F_3, it is possible to obtain reliable ideas about the nature of inheritance of such characters.

1. Maturity as measured by the date of heading.

Nilsson-Ehle [1908] found transgressive segregation in a cross between medium early and late maturing oat varieties. Caporn's results [1918] indicated that three factors were probably involved. Noll [1925] found earliness to be due to a series of dominant factors with a cumulative effect while Garber and Quisenberry's results [1928] show that early ripening is dominant and that only two factors are involved.

Table XVII shows the frequency distribution of the number of days taken to head out by the parents, F_1, and F_2 and some F_3 families in the crosses under discussion. The average number of days taken by Scotch Potato to show the first flower was $137{\cdot}40 \pm 0{\cdot}54$ days in 1928 and $138{\cdot}16 \pm 0{\cdot}56$ days in 1929 respectively, and indicated that the variety was extremely late. Abundance followed quite closely with an average of $131{\cdot}33 \pm 0{\cdot}42$ days in 1929 and $136{\cdot}19 \pm 0{\cdot}27$ days in 1930 and was similarly quite late. B. S. 4 headed out in $76{\cdot}36 \pm 0{\cdot}45$ days in 1928 and $78{\cdot}21 \pm 0{\cdot}26$ days in 1929 and $88{\cdot}07 \pm 0{\cdot}22$ days in 1930. This was the early parent in Crosses I and III, while the early parent in Cross II was B. S. 2 with $85{\cdot}76 \pm 0{\cdot}43$ days in 1928 and $84{\cdot}00 \pm 0{\cdot}41$ days in 1929. Differences between the exotic and Pusa parents were therefore statistically significant.

TABLE XVII.

Frequency distribution showing the number of days taken to head out by plants in oat crosses and their parents.

Cross No.	Family	Year	Class centres showing number of days taken to head out																				Total number of plants	Mean	S. Dev.	Coefficient of variation
			F_2 parent	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150				
I	Scotch Potato parent	1928	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	14	9	19	5	3	50	137·40±0·54	5·75±0·38	4·18±0·28
	Do.	1929	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	5	33	45	3	5	93	138·16±0·56	8·10±0·40	5·86±0·28
	B. S. 4.—parent	1928	..	..	1	11	33	15	8	3	..	..	..	..	..	..	..	..	..	..	..	..	..	71	76·36±0·45	5·65±0·32	7·39±0·41
	Do.	1929	..	..	..	5	43	38	2	6	1	..	..	..	..	..	..	..	..	..	..	..	..	95	78·21±0·26	3·81±0·18	4·87±0·23
	Scotch Pot. × B. S. 4-F_1.	1927	.	..	..	×	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	72·00	..	..
	Scotch Pot. × B. S. 4-F_2.	1928	..	..	..	..	18	16	61	49	66	24	44	14	1	6	3	5	..	..	..	..	..	307	94·80±0·43	11·10±0·30	11·70±0·31
	I-201—F_2 . .	1929	73	..	..	3	37	26	18	7	2	..	..	..	..	..	..	..	..	..	..	..	..	93	79·78±0·40	5·75±0·28	7·20±0·35
	I-139—F_2 . .	1929	84	..	..	1	4	27	34	22	1	4	..	..	..	..	..	..	..	..	..	..	..	93	84·95±0·35	5·00±0·24	5·88±0·29
	I-140—F . .	1929	85	..	..	2	27	42	8	8	0	0	1	..	..	..	..	..	..	..	..	..	..	88	79·88±0·41	5·70±0·29	7·13±0·36
	I-270—F_2 . .	1929	89	..	..	..	3	9	13	22	7	7	5	2	..	..	..	..	..	..	..	..	..	68	90·30±0·65	8·05±0·46	8·91±0·51
	I-104—F_2 . .	1929	80	..	..	6	7	24	14	8	1	0	6	1	1	5	10	1	0	1	..	..	85	92·30±1·24	17·00±0·88	18·41±0·95
	I- 10—F_2 . .	1929	88	..	..	1	2	11	9	23	3	8	1	7	0	2	2	4	0	7	..	..	80	98·64±1·41	18·70±1·00	18·95±1·01
II	Scotch Potato parent	1928	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	14	9	19	5	3	50	137·40±0·54	5·75±0·38	4·18±0·28
	Do. .	1929	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	5	33	45	3	5	93	138·16±0·56	8·10±0·40	5·86±0·28
	B. S. 2.—parent .	1928	..	..	..	..	2	24	29	21	6	3	1	..	..	..	..	..	..	..	..	..	..	86	85·76±0·43	6·00±0·31	6·99±0·35
	Do. .	1929	..	..	..	..	3	44	35	7	4		0	3	..	..	..	..	..	..	..	..	..	97	84·00±0·41	6·19±0·29	7·26±0·35
	Scotch Pot. × B. S. 2-F_1.	1927	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	73·00	..	..
	Scotch Pot. × B. S. 2-F_2.	1928	..	..	..	2	8	21	36	56	58	37	19	12	8	7	6	6	3	2	..	..	281	96·18±0·52	12·90±0·36	13·41±0·38

	II-151—F_3	1929	75	..	..	2	16	47	22	4	5	..	..	..	..	..	..	..	..	..	..	..	96	81·30±0·35	5·12±0·24	6·29±0·30
	II- 97—F_3	1929	80	..	..	1	7	43	19	7	4	0	1	..	..	..	..	..	..	..	..	..	92	81·66±0·39	5·55±0·27	6·79±0·33
	II-246—F_3	1929	80	..	1	1	10	33	37	8	3	3	..	..	..	..	..	..	..	..	..	..	96	83·20±0·40	5·90±0·28	7·09±0·34
	II- 52—F_3	1929	84	..	..	..	7	26	25	16	17	2	2	..	..	..	..	..	..	..	..	..	95	86·25±0·48	6·98±0·34	8·09±0·39
	II-112—F_3	1929	84	..	..	2	7	24	7	20	14	5	3	0	1	2	0	0	5	5	..	..	95	93·20±1·31	19·00±0·92	20·38±0·99
	II-183—F_3	1929	90	..	..	..	..	..	1	9	17	30	1	12	9	1	0	3	5	3	1	..	92	105·65±0·97	13·80±0·68	13·06±0·64
III	Abundance parent	1929	..	..	..	..	..	..	..	..	..	..	..	..	..	2	12	24	19	7	..	..	64	131·33±0·42	5·07±0·30	3·86±0·23
	Do.	1930	..	..	..	..	..	..	..	..	..	..	..	..	..	2	26	69	33	11	3		144	136·19±0·27	4·74±0·19	3·48±0·32
	B. S. 4—parent	1929	..	..	..	5	43	38	2	6	1	..	..	..	..	..	..	..	..	..	..	..	95	73·21±0·26	3·81±0·18	4·87±0·23
	Do.	1930	..	..	..	..	5	25	75	47	10	1	..	..	..	..	..	..	..	..	..	..	163	88·07±0·22	4·21±0·16	4·78±0·45
	Abundance × B. S. 4-F_1.	1928	..	..	..	..	..	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	2	93·00	..	..
	Abundance × B. S. 4-F_2.	1929	..	..	1	8	15	71	29	61	30	16	1	2	4	9	27	9	5	6	..	..	294	94.75±0·71	18·09±0·50	19·09±0·53
	III-265—F_3	1930	72	2	16	14	10	8	12	1	..	..	..	..	..	..	..	..	..	..	..	..	63	73·65±0·67	7·87±0·47	10·68±1·04
	III- 6—F_3	1930	78	2	10	19	11	9	8	10	1	..	..	..	..	..	..	..	..	..	..	..	70	76·00±0·71	8·87±0·95	11·66±1·05
	III- 59—F_3	1930	80	..	..	1	24	24	1	15	0	9	..	..	..	..	..	..	..	..	..	..	83	81·39±0·66	8·86±0·46	10·67±0·91
	III-296—F_3	1930	85	..	..	3	15	12	12	27	2	3	..	..	..	..	..	..	..	..	..	..	74	84·25±0·57	7·31±0·40	8·68±0·78
	III-249—F_3	1930	105	..	..	..	..	3	5	10	2	17	4	13	3	2	2	5	1	..	..	..	67	103·35±1·12	13·65±0·80	13·24±0·89
	III-268—F_3	1930	105	..	..	..	7	4	0	9	2	12	0	6	6	4	5	5	1	..	..	..	61	103·05±1·56	18·03±1·10	17·50±1·07

F_1 plants in Crosses I and II showed dominance of earliness and headed out 72 days after seeding. In Cross III however the F_1 plant was intermediate in maturity and took 98 days.

The F_2 in the first two Crosses with Scotch Potato oats showed transgressive segregation, the population being distributed in a skew curve with the mode towards the early parent, and a coefficient of variability much higher than that of either parents. This is depicted in Fig. 4. Owing to the fact that a number of very late plants are dried up by the west winds before flowering, the full range of the later parent is not realised in F_2.

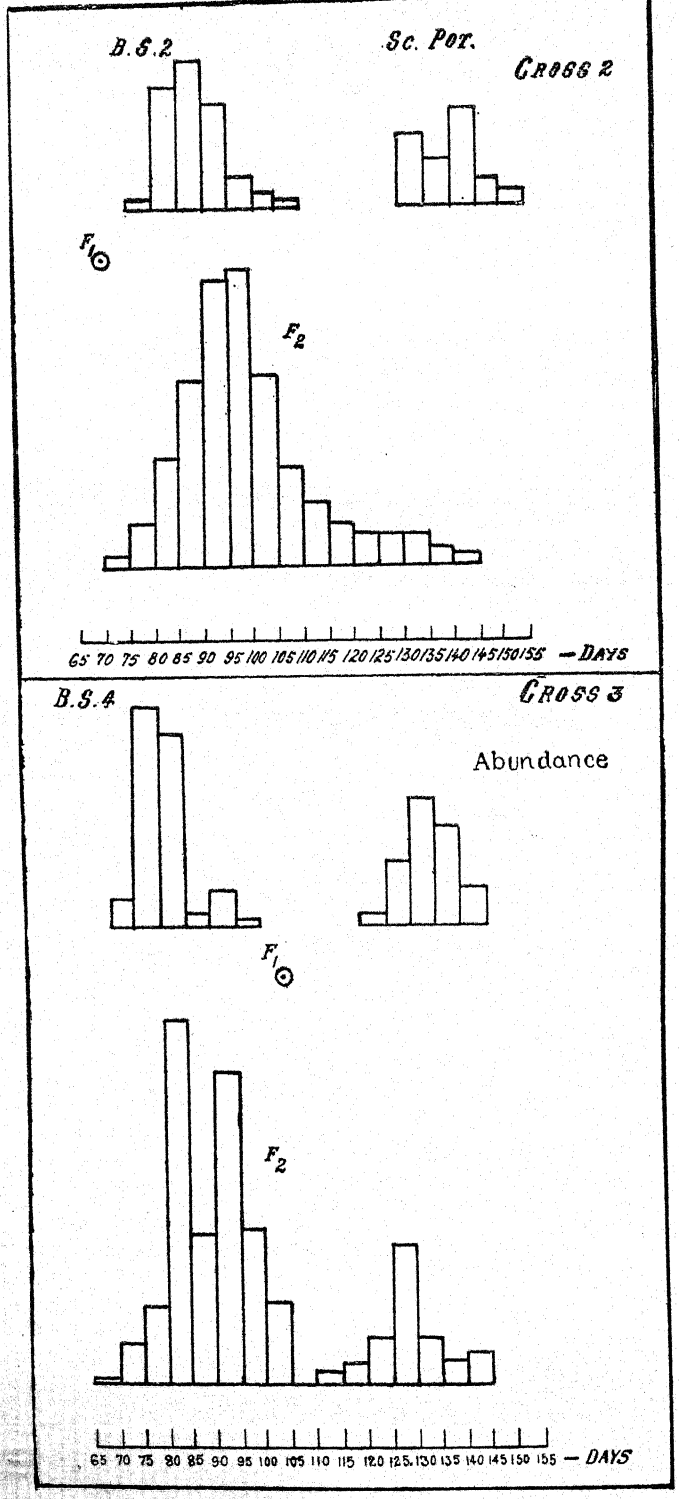


Fig. 4. Inheritance of maturity in oats showing frequency distributions of the number of days taken to head out.
Cross 2.—(Top figure) showing segregation on multiple-factor basis.
Cross 3.—(Lower figure) showing segregation on a single-factor basis.

In Cross III with Abundance oats in which the F_1 plant was intermediate between both parents, the F_2 exhibited a bimodal curve (Fig. 4) and extended over the range of both parents, the modes coinciding more or less with those of the parents. The frequencies of the F_2 population within each parental range are given below and suggest a monohybrid type of segregation with simple dominance of earliness :—

TABLE XVIII.

Segregation in F_2 of early and late maturing plants on a 3 : 1 ratio in Cross III.

Cross	Frequency	Maturity		Total number of plants	Dev.	P. E.	$\frac{\text{Dev.}}{\text{P. E.}}$
		Early	Late				
III	Observed	232	62				
	Expected	220·5	73·5	294	11·5	5·01	2·29

In all the crosses selections for F_3 were strongly influenced by the fact that late flowering is an economic disadvantage for Indian conditions. Such selections were therefore restricted to the more early-maturing individuals and as a result none of the F_3 families exhibited lateness.

2. Height of plants.

Nilsson-Ehle (1908) crossed two *sativa* varieties of oats which differed in height and obtained transgressive segregation in F_2. A similar result was obtained by Surface [1916] in a cross between *A. fatua* (tall) × *A. sativa* var. Kherson (low). These results may be explained by the assumption of multiple genes.

The height of plants is a variable character as it is greatly influenced by soil and climatic conditions. This may be seen from the average heights of Scotch

Potato, B. S. 2 and B. S. 4 oats during the three consecutive years, 1927 to 1929 :—

Type	Average heights in cm.		
	1927	1928	1929
Scotch Potato oats	90·0	87·84	104·76
B. S. 4 oats	90·0	129·42	144·36
B. S. 2 oats	85·0	122·13	150·21

In Crosses I and II the F_1 individuals were grown in 1927-28 and were 90 cm. and 88 cm. respectively in height. This was a year of low heights and hence due allowance must be made when comparing the F_1 figures with those of F_2 in Table XVIII which shows the frequency distributions of the height of plants in different generations. The following year, 1929, again showed greater increase in all parent forms and this was likewise manifested by many of the F_3 families studied during this year. On the other hand 1930 was again a year of low heights, as will be seen from the distributions of the heights of B. S. 4 and Abundance oats as well as those of the F_3 families of Cross III.

The differences between the F_3 means from their respective F_2 values for height are therefore due mainly to climatic variations.

The F_1 plants in all the three crosses therefore indicate no dominance but show a more or less intermediate nature while the F_2 progenies showed practically mid-parental heights and much larger variability than any of the parental types (Fig. 5). Transgressive segregation and the distribution of the heights almost approaching a normal frequency curve confirms the assumption of multiple factors for this character as is suggested also by other workers elsewhere.

Examples of some homozygous and some heterozygous F_3 families are shown in Table XVIII.

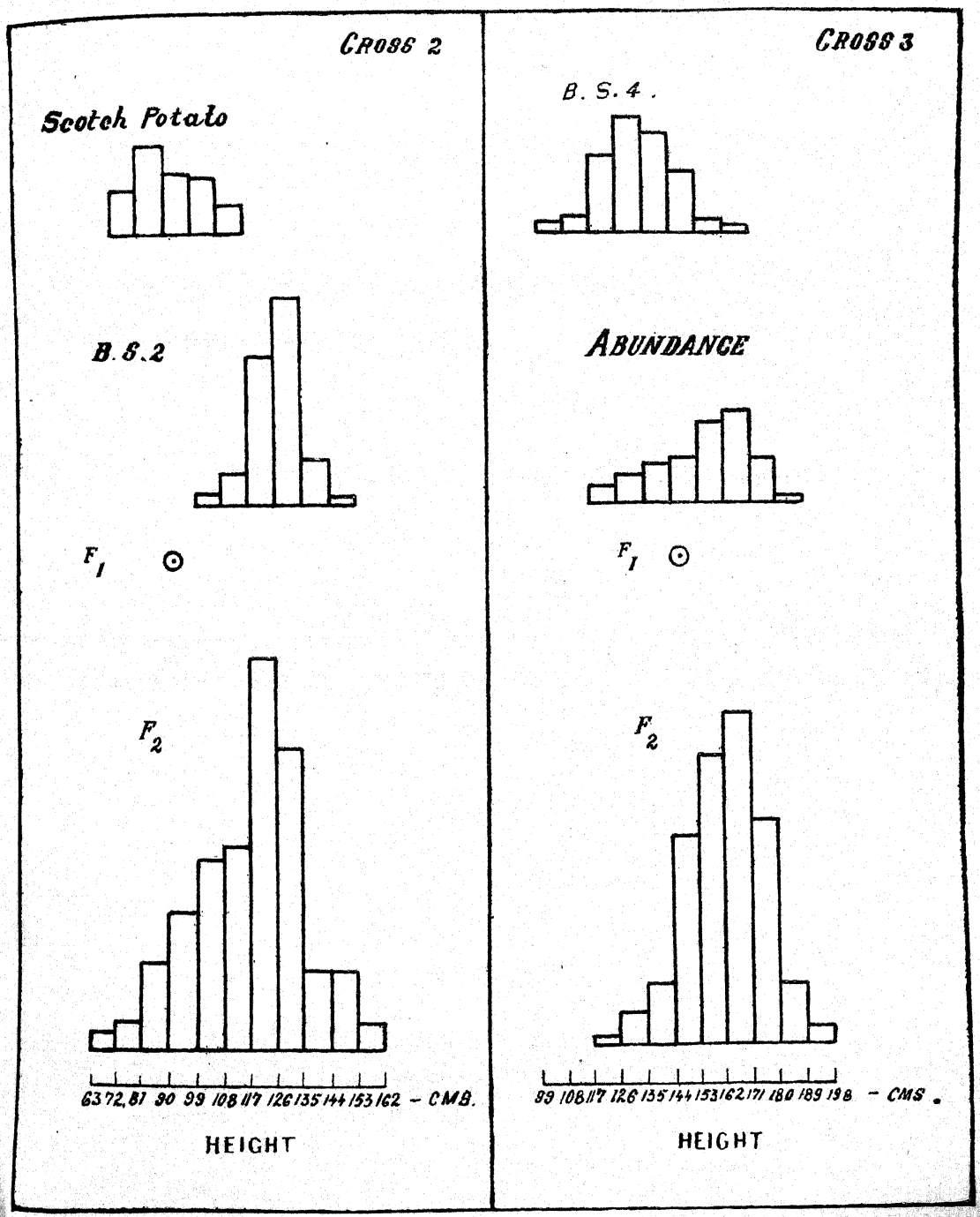


Fig. 5. Inheritance of height of plants in oats. Cross II (Left) and Cross III (Right). Absence of dominance and segregation on multiple-factor basis is evident.

TABLE XVIII.

Frequency distribution showing the height of plants in oat hybrids and their parents.

Cross No.	Family	Year	Class centres in cm.															Total number of plants	Mean	S. Dev.	Co-efficient of variation
			F_2 parent	63	72	81	90	99	105	117	126	135	144	153	162	171	180	189				
I	Scotch Potato parent	1928	..	..	8	16	11	10	5	..	..	..	..	..	..	..	..	..	50	87·84±1·04	10·98±0·74	12·50±0·84
	Do.	1929	..	..	..	3	4	32	40	11	1	1	..	..	..	..	..	..	92	104·76±0·62	8·82±0·43	8·42±0·41
	B. S. 4 parent	1928	..	..	..	..	..	1	3	14	21	18	11	2	1	..	..	..	71	129·42±0·93	11·71±0·66	9·11±0·51
	Do.	1929	..	..	..	..	..	..	..	..	1	19	53	21	2	..	..	..	96	144·36±0·45	6·66±0·32	4·61±0·22
	Scotch Potato × B.S. 4-F_1	1927	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	..	1	90·00	....	....
	Do. F_2	1928	..	..	2	11	12	19	56	56	69	47	26	7	1	1	..	..	307	119·79±0·65	17·10±0·46	14·27±0·38
	I-251—F_3	1929	120	..	..	..	..	1	8	30	35	10	3	..	..	..	..	..	87	122·58±0·62	8·64±0·44	7·04±0·35
	I-84—F_3	,,	134	..	..	..	..	..	..	..	3	21	47	17	3	..	..	..	91	143·64±0·52	7·36±0·36	5·12±0·25
	I-59—F_3	,,	135	..	..	..	..	..	..	..	3	11	26	25	7	1	..	..	73	146·07±0·71	9·09±0·50	6·22±0·34
	I-36—F_3	,,	137	..	..	..	..	..	..	1	6	14	25	31	10	..	..	..	87	146·25±0·77	10·71±0·54	7·32±0·37
	I-250—F_3	,,	122	..	..	..	1	3	2	9	13	13	3	8	1	..	..	..	53	130·05±1·43	15·49±1·01	11·91±0·78
	I-14—F_3	,,	134	..	..	1	2	2	1	7	13	15	21	6	1	..	..	..	69	132·66±1·31	16·21±0·93	12·21±0·70
II	Scotch Potato parent	1928	..	..	8	16	11	10	5	..	..	..	..	..	..	..	..	..	50	87·84±1·04	10·98±0·74	12·50±0·84
	Do.	1929	..	..	..	3	4	32	40	11	1	1	..	..	..	..	..	..	92	104·76±0·62	8·82±0·43	8·42±0·41
	B. S. 2 parent	1928	..	..	..	..	..	2	6	27	38	8	1	..	..	..	..	..	82	122·13±0·60	8·15±0·42	6·67±0·35

| | | |

3. Number of spikelets per panicle.

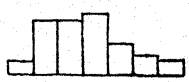
Nilsson-Ehle [1908] has reported the occurrence of transgressive segregation again in the inheritance of the number of florets in the spikelet indicating that the character owed its expression to several genes.

In Crosses I and II with the Scotch Potato oats the F_2 and F_3 populations show a higher spikelet number than either parent. In Cross III however when one of these B. S. parents was crossed with the Abundance oats, the F_2 segregation is within the range of the parents. It appears therefore that the increase in spikelet number in the first two crosses was due to some factors present in the Scotch Potato variety. Table XIX shows the frequency distributions for this character in all the three crosses.

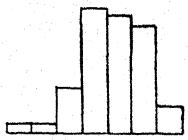
The Scotch Potato oat showed a mean of $83{\cdot}55 \pm 1{\cdot}15$ spikelets per panicle in a population of 111 plants in 1928 while the following year this type showed an average of $94{\cdot}35 \pm 1{\cdot}33$ spikelets in 94 plants studied. B. S. 4 parent on the other hand showed a mean of $80{\cdot}28 \pm 0{\cdot}89$ and $80{\cdot}80 \pm 0{\cdot}85$ respectively in these two years. The F_1 plant in Cross I had a mean of 116 spikelets to the panicle. The F_2 progeny had a mean of $117{\cdot}75 \pm 1{\cdot}69$ spikelets but the range of variation was greatly extended and the standard deviation was over twice as great as those of the parents. The plants with high spikelet numbers had as much as three or four times the average number of spikelets in the two parental varieties. Presumably additive factors are responsible for this increase. The frequency array indicates an approach to a normal curve and is a definite indication of transgressive segregation. Although the parent races exhibited the same spikelet numbers, more or less they must have differed in their genetic factors for this character. As a consequence of the production of new genetic combinations for spikelet number in F_2, new types appeared with higher and lower numbers than those of the parents (Fig. 6). These results are, of course, to be expected on a multiple-factor hypothesis and resemble those obtained by Hayes [1912] in the inheritance of leaf-number in tobacco. The frequency distribution, as well as the realization of plants in the extreme classes, indicates that only three or four factors are responsible for this character.

CROSS II

Sc. Pot.

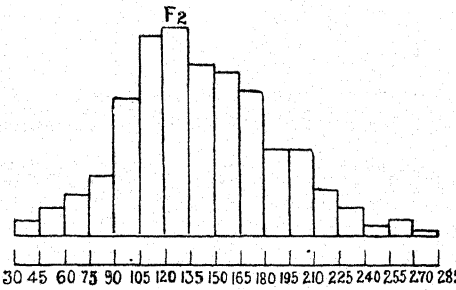


B.S.2.



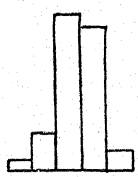
⊙F1

F2

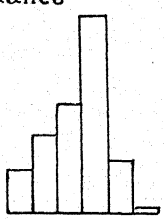


B.S.4.

CROSS III



Abundance



⊙F1

F2

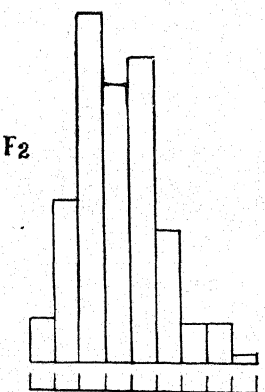


TABLE XIX.

Frequency distribution showing the number of spikelets per panicle in oat plants in hybrids and their parents.

Cross No.	Family	Year	Class centres of average number of spikelets per panicle																					Total number of plants	Mean	S. Dev.	Co-efficient of variation
			F_2 parent	15	30	45	60	75	90	105	120	135	150	165	180	195	210	225	240	255	270	285	300				
I	Scotch Potato parent	1928	..	..	..	11	11	12	60	14	3	..	..	..	..	..	..	..	..	..	..	..	..	111	83·55±1·15	18·04±0·82	21·60±1·02
	Do.	1929	..	..	1	4	20	32	22	9	5	1	..	..	..	..	..	..	..	..	..	..	..	94	94·35±1·33	19·26±0·94	20·41±1·00
	B. S. 4 parent	1928	..	..	..	2	7	30	28	4	..	..	..	..	..	..	..	..	..	..	..	..	..	71	80·28±0·89	11·20±0·63	13·95±0·78
	Do.	1929	..	..	..	1	10	45	31	9	..	..	..	..	..	..	..	..	..	..	..	..	..	96	80·80±0·85	12·45±0·60	15·40±0·74
	Scotch Potato ×B.S. 4-F_1	1927	..	..	..	..	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	..	..	1	116·00	..	..
	Scotch Potato ×B.S. 4-F_2	1928	..	1	5	9	17	39	39	39	39	34	33	19	10	10	6	2	4	1	..	..	..	307	117·75±1·69	44·10±1·20	37·45±1·01
	I-59—F_3	1929	145	..	..	..	..	2	8	13	21	16	8	7	2	..	..	..	..	..	..	..	..	77	124·95±1·86	24·15±1·31	19·33±1·05
	I-140—F_3	1929	146	..	1	2	1	4	7	24	14	13	11	4	5	..	..	..	..	..	..	..	..	86	119·40±2·25	30·90±1·59	25·04±1·29
	1-14-A—F_3	1929	202	..	..	..	..	..	2	3	12	7	14	17	10	12	1	1	3	..	..	..	..	82	161·70±2·16	28·95±1·52	17·90±0·94
	I-300—F_3	1929	240	..	..	..	..	..	..	..	1	1	8	3	7	8	25	9	11	8	3	1	..	85	194·55±2·53	34·64±1·79	17·81±0·92
	I-273—F_3	1929	152	..	..	..	6	2	0	4	7	6	8	6	6	8	9	4	4	1	1	1	..	74	164·25±4·19	53·40±2·96	32·80±1·82
	I-321—F_3	1929	172	..	..	..	..	2	1	6	14	10	7	9	11	5	6	7	3	2	3	1	..	87	167·25±3·45	48·75±2·44	28·39±1·02
II	Scotch Potato parent	1928	..	..	..	11	11	12	60	14	3	..	..	..	..	..	..	..	..	..	..	..	..	111	83·55±1·15	18·04±0·82	21·60±1·02
	Do.	1929	..	..	1	4	20	32	22	9	5	1	..	..	..	..	..	..	..	..	..	..	..	94	94·35±1·33	19·26±0·94	20·41±1·00
	B. S. 2 parent	1928	..	..	..	2	2	9	24	23	21	5	..	..	..	..	..	..	..	..	..	..	..	86	85·65±1·41	19·50±1·00	22·76±1·17
	Do.	1929	..	..	..	..	1	12	21	26	36	4	..	..	..	..	..	..	..	..	..	..	..	100	104·40±1·15	17·05±0·81	16·33±0·77
	Scotch Potato ×B. S. 2-F_1	1927	..	..	..	..	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	..	..	1	120·00	..	..

	Scotch Potato × B. S. 2-F_3	1928	..	..	3	5	8	11	26	39	41	33	32	28	17	17	9	5	2	3	1	..	..	280	119·83±1·81	45·00±1·28	37·55±1·07
	II-246—F_3	1929	152	..	..	..	3	7	26	28	20	8	1	1	..	..	..	..	..	..	..	..	..	94	105·00±1·25	18·10±0·89	17·23±0·84
	II-338—F_3	1929	163	..	..	..	2	3	6	38	32	14	3	..	..	..	..	..	..	..	..	..	..	98	112·78±1·15	16·59±0·81	15·02±0·72
	II-93—F_3	1929	174	..	..	..	..	2	8	21	23	20	18	3	1	0	2	..	..	..	..	..	..	98	125·79±1·68	24·67±1·18	19·61±0·94
	II-8—F_3	1929	175	..	..	..	..	..	..	1	11	5	13	19	15	8	9	1	..	..	..	..	..	82	165·39±2·10	28·27±1·48	11·04±0·58
	II-312—F_3	1929	175	..	..	..	..	..	..	3	10	17	8	8	5	9	3	3	4	1	0	1	2	74	167·40±3·59	45·90±2·54	27·41±1·52
	II-97—F_3	1929	240	..	..	..	1	0	5	1	6	19	11	14	8	15	5	1	2	2	2	..	..	92	163·36±2·82	40·20±1·99	24·60±1·22
III	Abundance parent	1929	..	..	..	8	15	21	38	10	1	..	..	..	..	..	..	..	..	..	..	..	..	93	79·83±1·21	17·29±0·85	21·66±1·12
	Do	1930	..	..	..	6	4	17	68	20	4	..	..	..	..	..	..	..	..	..	..	..	..	119	88·09±0·94	15·15±0·66	17·20±0·77
	B. S. 4 parent	1929	..	..	..	1	10	45	31	9	..	..	..	..	..	..	..	..	..	..	..	..	..	96	80·80±0·85	12·45±0·60	15·40±0·74
	Do.	1930	..	..	..	10	78	64	20	1	..	..	..	..	..	..	..	..	..	..	..	..	..	173	77·55±0·72	13·99±0·51	18·04±0·67
	Abundance × B. S. 4-F_1	1928	..	..	..	..	..	..	..	×	..	..	..	..	..	..	..	..	..	..	..	..	..	2	98·00	..	..
	Abundance × B. S. 4-F_2	1929	..	..	..	9	32	69	55	60	26	8	8	2	..	..	..	..	..	..	..	..	..	269	91·05±0·98	23·90±0·69	26·24±0·76
	III-6—F_3	1930	37	6	34	21	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	65	35·25±0·92	11·10±0·65	31·48±1·86
	III-265—F_3	1930	46	1	13	36	8	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	62	45·15±1·05	12·36±0·74	27·37±1·65
	III-59—F_3	1930	51	1	12	26	23	5	4	1	..	..	..	..	..	..	..	..	..	..	..	..	..	72	52·20±1·37	17·30±0·97	33·14±1·86
	III-296—F_3	1930	64	..	1	22	18	15	5	1	..	..	..	..	..	..	..	..	..	..	..	..	..	62	60·75±1·19	15·00±0·84	24·69±1·38
	III-268—F_3	1930	80	..	..	7	9	14	16	11	4	1	..	..	..	..	..	..	..	..	..	..	..	62	82·50±1·88	22·00±1·33	26·66±1·61
	III-127—F_3	1930	80	2	1	5	12	13	8	5	4	1	1	0	1	..	..	..	..	..	..	..	..	53	78·60±2·88	31·10±2·03	39·56±2·59

Plants with high spikelet numbers were selected in the F_2 and their progeny in the F_3 yielded many families homozygous for this character and a number of heterozygous families. Due allowance, however, must be made for the fact that hybrid vigour in F_2 parental forms induced higher average spikelet numbers in these which were not absolutely realised to the same degree in their F_3 progenies.

In Cross II the same conditions obtained, Scotch Potato oat being the common parent in both these crosses.

Cross III on the other hand presented quite a different nature. Here the average number of spikelets per panicle in the Abundance oats was $79{\cdot}83 \pm 1{\cdot}21$ in 1929 and $88{\cdot}09 \pm 0{\cdot}94$ in 1930. B. S. 4 as shown in the previous cross had an average number of $80{\cdot}80 \pm 0{\cdot}85$ spikelets to the panicle in 1929 and $77{\cdot}55 \pm 0{\cdot}72$ spikelets per panicle in 1930. The F_1 showed about 98 spikelets. The F_2 progeny had a mean of $91{\cdot}05 \pm 0{\cdot}98$ and a standard deviation of only $23{\cdot}90 \pm 0{\cdot}69$. The frequency distribution when graphically represented (Fig. 6) shows a skew curve indicating a dominance of plants with a low spikelet number. The F_3 families unlike those in the first two crosses do not likewise show a high spikelet number.

Plate LXIV depicts the average number of spikelets per panicle in the Scotch Potato and B. S. 2 parents as well as in the F_2 hybrids from a cross between these two types of oats. Panicles with a large number of spikelets were ultimately selected for F_3 study.

XI. Discussion of results.

Qualitative characters.

It is evident from these studies that a unit factor difference is present in the type of floret separation or basal articulation in three crosses between *Avena sativa* L. $\times$*Azena sterilis, culta* L. The exotic parents, Scotch Potato and Abundance have a *sativa* type of base and may be represented by the factors **AA**. Both B. S. 2 and B. S. 4, on the other hand, possess a *sterilis* type of base and may be represented by the allelomorph **aa**. The F_1 and the other heterozygotes in the F_2 have an intermediate type of base and must therefore be **Aa**; the F_2 segregation being 1 **AA** : 2 **Aa** : 1 **aa**.

Similarly a monofactorial mode of inheritance has been observed between plants with strong awns (Scotch Potato and Abundance) and plants with "weak" awns (B. S. 2 and B. S. 4). If **BB** represents the factor for strong and **bb** for weak awns the F_2 segregation may be shown by 1 **BB** : 2 **Bb** : 1 **bb**.

Linkage between (a) the *sterilis* type of base and (b) *weak* awns has also been observed. This is very strong in the cross between Abundance and B. S. 4 oats, the cross-over value being only 3·3 per cent. In the other two crosses with Scotch Potato oats and B. S. 4 and B. S. 2 the cross-over values are 17 and 23 per cent. respectively.

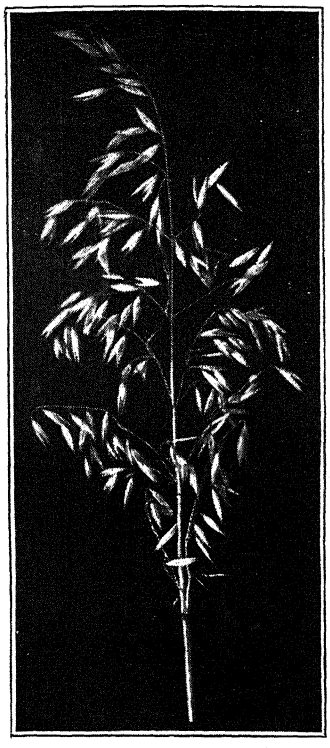
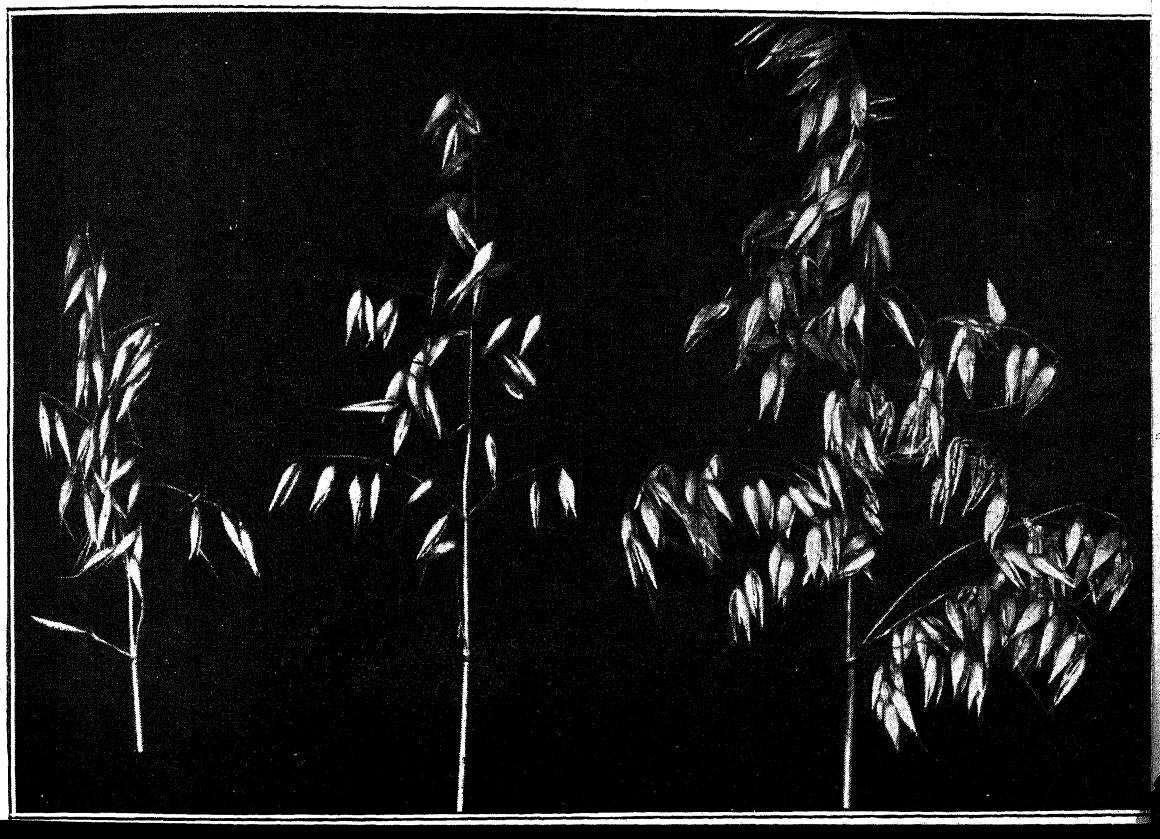
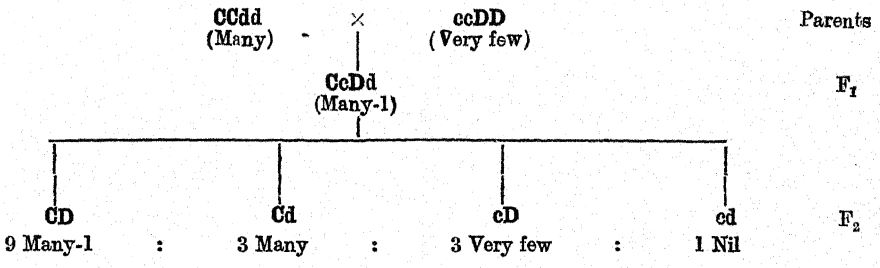


Fig. 1.—B. S. 2 parent.

Fig. 2.—S. P. parent.



For the inheritance of basal pubescence, however, at least two pairs of factors are responsible. If **C** be a factor for producing 'many' hairs and **D** be a factor for 'very few' hairs which also reduces the action of **C** and, when present with it, reduces the extent of hairiness to 'many-1', the B. S. types 2 and 4 may be represented by **CCdd** and the Scotch Potato and the Abundance oats by **ccDD**. It will be evident that the presence of both **C** and **D** together would produce 'many-1' hairs (lesser in extent than 'many' hairs). The complete absence of both these factors for hairiness on the other hand would produce another new phenotype 'nil' which would have no hairs at the base of the grain. Thus :—



The following table shows the genotypes and phenotypes observed in F_2 as well as the F_3 expectations :—

TABLE XX.

Genotypes and phenotypes observed in F_2 and the theoretical expectations in F_3.

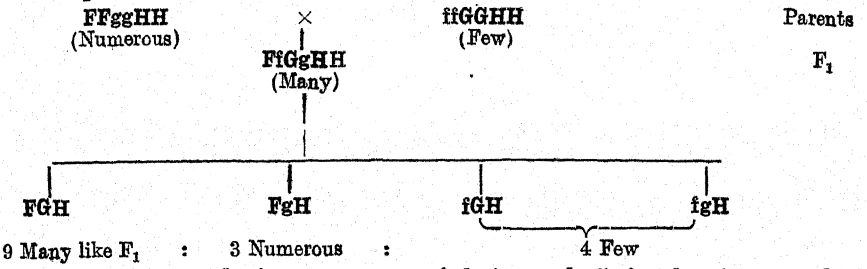
Genotypes	F_2 phenotypes	F_3 expectations
1 **CCDD**	Many-1	Pure breeding
2 **CCDd**	,,	3 Many-1 : 1 Many but difficult to differentiate from above (page 786)
2 **CcDD**	,,	3 Many-1 : 1 Very few
4 **CcDd**	,,	9 Many-1 : 3 Many : 3 Very few : 1 Nil
1 **CCdd**	Many	Pure breeding
2 **Ccdd**	,,	3 Many : 1 Nil
1 **ccDD**	Very few	Pure breeding
2 **ccDd**	,,	3 Very few : 1 Nil
1 **ccdd**	Nil	Pure breeding

In the study of the F_2 generation the 'very few' and 'nil' phenotypes were combined at first and contrasted with the 'many-1' and 'many' phenotypes, as it was believed that segregation was on a monohybrid basis giving 1 'very few' : 3 'many' hairs. The behaviour of the F_3 generation, however, proved the fallacy of this assumption and it was found that two factors were responsible for the inheritance of this character and the true nature of segregation was as indicated above.

A few F_3 families were studied also for the size of basal hairs in Cross III and these suggest that a single factor is also responsible for the inheritance of this

character and that this factor is quite independent of the factors for the production of basal hairs.

In the Scotch Potato oats the margin of the leaves is beset with 'numerous' fine hairs while in B. S. 2 and B. S. 4 oats there are only a 'few' hairs restricted mainly to the lower one-third of the leaf. The F_1 plants in Crosses I and II have 'many' hairs, more in extent than the B. S. parents but distinctly lesser than those in the Scotch Potato parent. The 'many' phenotype behaved as the double dominant in the F_2, the nature of inheritance being as follows :—



Here **F** is the factor for producing 'numerous' hairs and **G** is the factor which reduces the action of **F**, while **H** is the basic factor for producing leaf-hairs. So that whenever **F** and **G** are present together the leaf-hairs are reduced to 'many' like F_1.

The Abundance oat has almost no hairs on the leaf-margin and so the nature of inheritance of leaf-hairs has not been studied in Cross III.

Quantitative characters.

Multiple factors are involved in the inheritance of early and late maturing plants in Crosses I and II, while in Cross III a clean-cut segregation in the F_2 into approximately three-fourths early and one-fourth late plants suggests a monohybrid mode of inheritance.

Multiple factors are again responsible for the inheritance of spikelet number per panicle in Crosses I and II. The F_2 generation presented some plants with lower and some with enormously higher spikelet numbers than either parents, although both the parents exhibited approximately the same number of spikelets per panicle. Additive factors are perhaps responsible for this increase in number and a curve of the error type given by the F_2 population shows that transgressive segregation is present here. Cross III however presents a skew curve for the F_2 and indicates dominance of plants with low spikelet numbers.

Transgressive segregation and the distribution of heights of plants in the form of a normal frequency curve suggests the presence of multiple factors again for the inheritance of the height of plants in the oat crosses under consideration. This character, it must be remembered, is greatly influenced by environmental conditions but its mode of inheritance is nevertheless brought out.

Summary.

Inheritance of some characters in three interspecific crosses between *Avena sativa* Linn. × *A. sterilis, culta* Linn. (*A. byzantina* Koch.) have been described. The oat crosses studied are :—

Cross I—Scotch Potato oats × B. S. 4
,, II— ,, ,, ,, × B. S. 2
,, III—Abundance ,, × B. S. 4

The Scotch Potato and the Abundance oats possess a *sativa* type of base and the B. S. oats have *sterilis* type of base.

Although reciprocal crosses were attempted in every case, success in crossing was only achieved with the late maturing *Sativa* varieties as the pistillate parent.

Single factor differences have been observed between :—

Sativa and *sterilis* types of base,
' strong ' and ' week ' awns,
and ' long' and ' short' basal hairs.

Two factors are responsible, on the other hand, for the inheritance of basal hairs and hairs on the margin of leaves.

Transgressive segregations indicating the presence of multiple factors have been observed in the inheritance of height of plants in all the three crosses, number of days taken to head out and the number of spikelets per panicle in Crosses I and II. While in Cross III dominance of early maturing plants and plants with a low spikelet number have been observed.

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INHERITANCE OF GRAIN LENGTH IN RICE (*ORYZA SATIVA* L.)*

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(With one text-figure)

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I. Introduction.

The existence of numerous varieties in rice has actuated many a worker to attempt at their classification into various groups. The difficulty to follow any particular scheme has been mainly due to the 'fluctuations' of the characters used in the classification. Kikkawa [1912], Graham [1913], Beale [1927] and Sethi [1930] have recognised that the metrical attributes of grain, namely, length, breadth and thickness are more constant than many of the vegetative characters and hence have employed them as the basis of their classification. They have distinguished the groups according to the length of grain in relation to its breadth. As the thickness of the grain does not show such wide variations as the length and breadth, the size of the paddy grain is expressed with tolerable accuracy by its length and breadth only. The size of kernel inside the husk follows almost exactly the variations of the size of the unhusked grain with very few exceptions, and hence the latter may be taken as an index of the kernel size.

* Paper read before the Association of Economic Biologists, Coimbatore.

The importance of the size of grain in international rice trade can not be overstressed. Some markets prefer a fine long rice as the famous 'Patna', while others prefer a fine round type. In addition to the preferences of markets for particular sizes, the chief factor contributing to the success of rice trade is 'uniformity'. This is of paramount importance, especially in milling trade, as a mixture of different sizes means either a good percentage of loss as broken rice or a high percentage of unhusked grains. The problem of the breeder is to evolve particular grades of strains of rice according to the local mills and also, to the needs of the controlling markets. There is thus the necessity for a knowledge of the inheritance and behaviour of the characters governing the size of rice, so that the evolution of strains according to the preferences may be possible.

II. Review of literature.

So far, references relate only to the monofactorial segregation between the short round type and the long and narrow type in rice. Lien Fang Chao [1928] has published that short spikelet (round shape) is a simple dominant to the long type. Ramiah *et al* [1931] have recorded that short round type (*sirumani* type of grain) was a simple dominant to the long narrow type. It was then surmised that the factor or factors responsible for spikelet length might be different from those responsible for shape, but might be closely associated. In wheat, Engledow [1920] found that the inheritance of glume length was of the form of 1 : 2 : 1 in the F_2, the F_1 being intermediate. The difficulties met with in the genetic analysis of metrical characters formed the subject of a separate investigation by Engledow [1922] in which the fluctuations were sought to be 'handicapped' or compensated with the establishments of correlations or ratios. In 'Cucurbita pepo', Sinnot [1931] has recorded that disk shape was a simple dominant to the sphere and suggested a single factor for shape and a number of independent factors for size or volume and the dimensions of the fruit were the result of the interaction of these factors.

III. Effect of environment.

Before analysing the genetical factors controlling the size of grain, it is necessary to know how far environmental conditions affect the expression of size. Characters as height, flowering duration and tillering in rice are subject to a great deal of 'fluctuation', due to changes in environmental conditions as soils, manures and seasons. Considering the behaviour of all these characters, the size of grain may be considered to be least affected. And it is this relative constancy of the character under varied conditions that makes the classification of rices on the basis of grain size possible.

As mentioned before, thickness of the grain does not show considerable differences as the other two dimensions in the various types of varieties; the measurements of length and breadth express the relative size with good precision. The length and breadth measurements are recorded with the help of a slide caliper with a vernier scale reading up to 0·1 of a mm. There may be a slight difficulty in correctly judging the width of the grain, but the maximum width is always recorded, which can be done with ease after a slight experience. Lord [1929] has used a glass scale reading up to 0·5 mm., but the vernier calipers used here in recording dimensions of grains is safely the best for easy, quick and accurate measurements. He has concluded that a minimum of 20 grain measurements is necessary for a representative sample. To arrive at the size of a sample to be measured in our work at Coimbatore, a large number of samples from a pure line was measured, and the variability of grain length determined for the different sizes of samples (Table I).

TABLE I.

Variability of grain length in different samples (T. 25).

6 GRAINS			12 GRAINS			24 GRAINS			36 GRAINS		
Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.	Mean	S. D.	C. V.
7·2	0·20	2·8	7·2	0·26	3·6	7·3	0·27	3·7	7·4	0·31	4·2
7·3	0·29	3·9	7·5	0·23	3·1	7·3	0·35	4·7	7·4	0·28	3·8
7·4	0·22	3·0	7·3	0·39	5·4	7·5	0·25	3·3	7·4	0·27	3·6
7·4	0·32	4·2	7·3	0·35	4·8	7·4	0·27	3·7	7·3	0·33	4·5
7·2	0·30	4·2	7·5	0·21	3·0	7·3	0·31	4·3	7·3	0·25	3·4

The above table shows that a six-grain sample compares fairly with a 36-grain sample, and hence the measurements were restricted to six grains in each case. The grains taken for measurement from the earhead of the plant are either from the top or middle of it, as otherwise, there is the possibility of including half-set and ill-filled grains which are usually situated at the base of the panicle. It has also been found that grains in the different tillers of the sample plant, do not vary significantly in size.

As regards the effect of soil on grain size, it is usually the complaint of cultivators that fine varieties get coarser gradually during years of cultivation in heavy delta soils. There appears to be a certain amount of truth in this complaint as Sreenivasan [1932] has observed that in some of the types sent to Maruteru in Godavari Delta from Coimbatore, the grains are gradually becoming coarser (Tables

II and II*a*). But detailed investigations are necessary to properly sassess the rôle of soil conditions on the modification of grain size.

TABLE II.

Variation in grain size of two types sent from Coimbatore and grown in Maruteru from 1925 to 1928.

	Years	Length in mm. (Mean of 100 grain sample)	Breadth in mm. (Mean of 100 grain sample)
T. 330	1925-26 (Original seed from Coimbatore).	5·46 ± ·02	2·58 ± ·014
	1926-27	5·54 ± ·02	2·70 ± ·011
	1927-28	5·64 ± ·02	2·73 ± ·012
	1928-29	5·99 ± ·02	2·82 ± ·008
T. 314	1925-26 (Original seed) . .	8·16 ± ·049	....
	1926-27	8·53 ± ·038	....
	1927-28	8·63 ± ·046	....
	1928-29	8·76 ± ·039	....
	1929-30	8·67 ± ·036	....

TABLE II*a*.

Variation in grain size of the same types grown in Coimbatore.

	Years	Length in mm. (Mean of 6 grain sample)	Breadth in mm. (Mean of 6 grain sample)
T. 330	1925-26	5·50 ± ·03	2·60 ± ·02
	1926-27	5·45 ± ·04	2·60 ± ·03
	1927-28	5·72 ± ·03	2·73 ± ·03
T. 314	1925-26	8·20 ± ·09	....
	1926-27	8·30 ± ·12	....
	1927-28	8·50 ± ·08	....

The size of grain obtained from a crop grown in different months of the same year under Coimbatore conditions does not show a big variation, although in sowings of certain months the grain size difference appears to be significant (Table III).

TABLE III.

Variation in length and breadth of grain in a crop of Co. 3 raised in different months in one year at Coimbatore.

Sowings	Length in mm. (100 grain sample)	Breadth in mm. (100 grain sample)
July	8·41 ± ·03	2·69 ± ·014
August	8·49 ± ·03	2·57 ± ·013
September	8·27 ± ·06	2·54 ± ·014
October	8·27 ± ·03	2·55 ± ·011
November	8·16 ± ·03	2·52 ± ·011
December	8·01 ± ·04	2·49 ± ·015

The effect of manuring and spacing on grain size in rice has not been studied fully, but it is considered that they do not have any, though findings are to the contrary in wheat [Engledow, 1930]. Ratooning of paddy, which is practised very occasionally in some parts of the Presidency, has a profound effect in reducing the size [John, 1927]. Thus it can be taken, that for stabilised types which have been grown in pure cultures for a number of years in the same place under identical conditions, the comparison of grain size in F_1, F_2 and F_3 cultures would not have been vitiated by growing these in different years. Sufficient precaution was however taken to see that the parents were grown at every stage side by side with the progenies of crosses.

IV. INHERITANCE STUDIES.

It had been established previously that in a cross between a long grain and a short round grain, the latter was a simple dominant over the former. But it could not be said definitely, whether it was not a possible association between the short length and the round shape, that was not responsible for this unifactorial inheritance. To get additional information on the point, a new cross was made between two types, T. 55 and T. 293, which had grains similar in shape but differed in length

by nearly 5 mm. The grain measurements of the two parents, F_1 and F_2, and the variability are given in Table IV.

TABLE IV.

Frequency distribution of grain length in parents and F_2 of the Cross T.55 × T.293.

Length in mm.	T.55 parent	T. 293 parent	F_1	F_2, 10329
5·7	4	..	..	..
5·9	17	..	..	..
6·1	11	..	..	2
6·3	..	..	..	2
6·5	..	..	..	3
6·7	..	..	..	9
6·9	..	..	..	13
7·1	..	..	..	20
7·3	..	..	..	29
7·5	..	..	..	38
7·7	..	..	..	32
7·9	..	..	..	45
8·1	..	..	..	17
8·3	..	..	..	14
8·5	..	..	..	6
8·7	..	..	..	4
8·9	..	..	..	5
9·1	..	..	..	9
9·3	..	..	..	7
9·5	..	..	..	10
9·7	..	..	..	10
9·9	..	..	..	26
10·1	..	..	..	23
10·3	..	..	..	19
10·5	..	2	..	15
10·7	..	4	..	9
10·9	..	5	..	7
11·1	..	10	..	1
11·3	..	14	..	1
11·5	..	2	..	3
11·7	..	1	..	1
Total No. of plants	32	38	..	380
Mean	5·95	11·11	7·7	8·53
Standard Deviation	0·13	0·27	..	1·31
Coefficient of variation	2·2±·19	2·4±·18	..	15·4±·37

It is seen that the F_1 is more or less intermediate between the parents and that the F_2 distribution covers the whole range of the parental limits in a definite bimodal form (Fig. 1). The range of the bigger mode is from 6·1 to 8·7 mm. and that of the smaller one from 8·7 to 11·7 mm., and the total number of plants on either side of the minimum frequency point is 234 and 146. Though the split is very clear these two classes could not be separated by the eye as was done in the case of the *anaikomban* and *sirumani* cross referred to by Ramiah [*loc. cit.*], where

the association of the round shape with a smaller spikelet length made the separation possible, by eye judgment only. All the 380 F_2 plants were grown in the following year to study this character in F_3s. One head per plant was collected from each of the F_3 family and made into separate bunches one for each family.

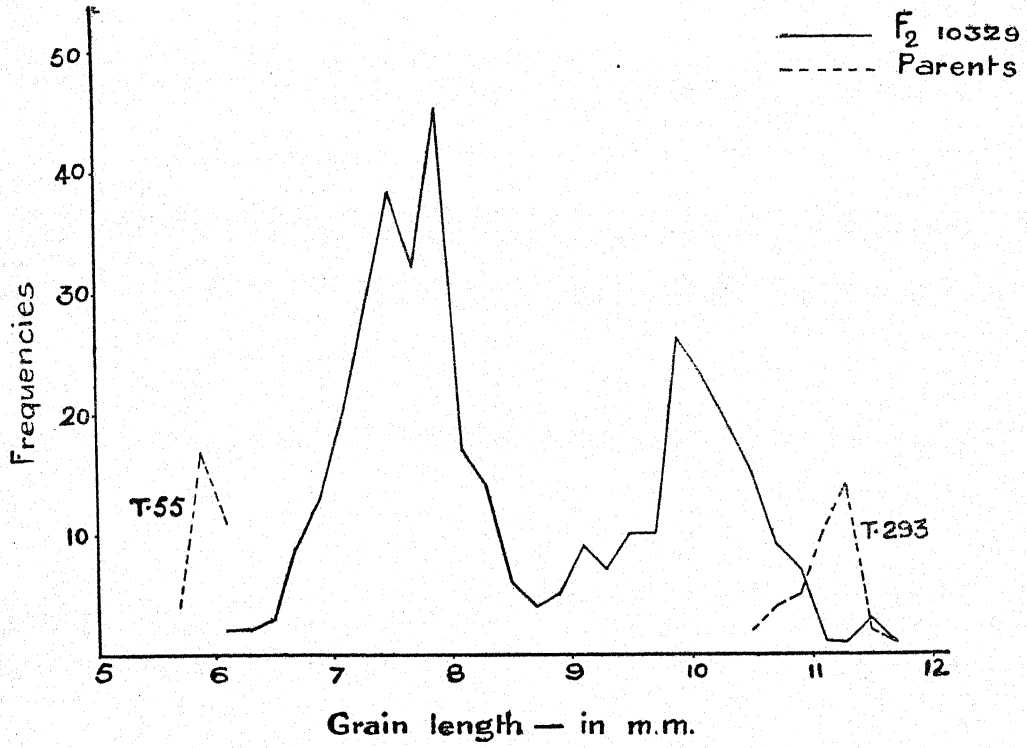

Fig. 1.—Range and distribution of grain length in parents and F_2.

These bunches were gone over in the laboratory to determine which of them was pure for grain size. The grain measurements of the apparent pure families were done, six grains in each panicle as before, and their means and variations determined. It was found that, in the majority of these pure families, there was a definite correspondence between the F_3 mean lengths and F_2 mean lengths, the difference between them not exceeding 0·2 mm. generally while in a few cases the difference was between 0·3 to 0·5 mm. The range, *i.e.*, the difference between the shortest and the longest grain in these pure families, was however slightly more than that observed for the two parents grown in the same season. The difference between the shortest and the longest grain varied from 0·9 mm. to as much as 1·6 mm. whereas the maximum difference for the parent never exceeded 1·1 mm. This might be either due to the phenomenon of 'shift' usually associated with the

inheritance of quantitative characters or to a number of minor subsidiary factors, besides the main important ones, as has been explained by Philiptschenko [1927]. For the families apparently segregating for spikelet length, a number were selected with means all along the F_2 range, and grain measurements recorded for the shortest and the longest spikelets in each of these families to determine the extent of F_3 range. A few of these splitting families were also studied individually for grain size variation. While such segregating families were found to occur all along the F_2 range, the range of spikelet length in their F_3s was never less than 2·2 mm. and in some cases it was as much as 4·2 mm. Details of measurements in a few of the typically pure and splitting families are given in Table V.

TABLE V.

A few segregating and pure families of the F_3. (Grain length in mm.)

Number	F_2 mean	F_3 mean	F_3 range	Standard Deviation	Coefficient of variation
Pure families					
(1) 11545	7·8	7·5 ± ·02	7·0 - 8·1	0·29	3·8
(2) 11906	9·0	8·8 ± ·03	8·2 - 9·8	0·40	4·5
(3) 11581	9·6	9·3 ± ·03	8·4 - 9·9	0·37	4·0
(4) 11615	9·9	9·9 ± ·02	9·4 - 10·4	0·25	2·6
(5) 11646	10·2	10·1 ± ·03	9·6 - 11·0	0·33	3·2
(6) 11638	10·5	10·0 ± ·02	9·5 - 10·5	0·22	2·2
Segregating families					
(1) 11579	7·1	7·8 ± ·07	6·4 - 10·0	1·2	15·3
(2) 11549	7·9	8·3 ± ·08	6·3 - 10·3	1·2	14·4
(3) 11570	8·0	8·0 ± ·07	6·7 - 10·8	1·0	12·9
(4) 11697	8·2	8·2 ± ·09	6·6 - 10·4	1·2	14·7
(5) 11659	8·2	8·0 ± ·08	7·0 - 10·1	0·9	11·7
(6) 11628	8·5	8·8 ± ·11	7·4 - 11·3	1·3	14·6
Parents					
T.55	5·9	5·9 ± ·02	5·5 - 6·1	0·14	2·3
T.293	11·1	10·7 ± ·03	10·2 - 11·3	0·25	2·3

The total number of F_3 families pure for grain length, 49 out of 380, are found to occur all along the F_2 frequency distribution (Table VI), and form about one-eighth of the total F_2 population.

TABLE VI.

Frequencies of pure and splitting F_3 families for grain length in mm.

F_2 Range	6·1	6·3	6·5	6·7	6·9	7·1	7·3	7·5	7·7	7·9	8·1	8·3	8·5	8·7	8·9
F_2 frequency	2	2	3	9	13	20	29	38	32	45	17	14	6	4	5
Pure for size	..	1	1	..	..	1	2	4	1	1	..	..	..	1	..
Splitting for size	2	1	2	9	13	19	27	34	31	44	17	14	6	3	5

F_2 Range	9·1	9·3	9·5	9·7	9·9	10·1	10·3	10·5	10·7	10·9	11·1	11·3	11·5	11·7	Total
F_2 frequency	9	7	10	10	26	23	19	15	9	7	1	1	3	1	380
Pure for size	4	2	..	2	10	3	5	6	2	2	..	..	1	..	49
Splitting for size	5	5	10	8	16	20	14	9	7	5	1	1	2	1	331

On a three-factor hypothesis, eight phenotype groups should occur in the ratios of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 in F_2, of which one in each group should breed pure in F_3, *i.e.*, there should be eight pure types out of the total sixty-four. This appears to agree well with the actual results obtained. Though it is not possible to determine the pure homozygous types in each of the eight phenotype groups, the phenotypes can be grouped together in various ways as 27 : 37 ; 36 : 28 and 36 : 9 : 19 ; and when the actual number of pure types obtained in each of these are compared with the theoretical number which should occur (Table VII), the deviations are found to be not significant.

TABLE VII.

Ratio of splitting to pure families in F_3=331 : 49. Theoretical ratio on a three-factor hypothesis=332·5 : 47·5. λ^2=·054. P=between ·9 and ·8.

Occurrence of pure families in the ratios group.

	27 : 37	*36 : 28*	*36 : 9 : 19*
Observed	10 : 39	11 : 38	11 : 7 : 31
Expected	6·7 : 42·9	12·3 : 36·7	12·3 : 6·1 : 30·6
λ^2 =	2·84	0·183	0·274
P=between	·10 and ·05	·70 and ·50	·90 and ·80

If shorter spikelets should be dominant to longer ones, and the number of Mendelian factors controlling length were more than one or two, the smaller grain size in F_2 must be associated with a larger number of factors, and hence the number of families breeding pure for short spikelet in F_3 must be comparatively few. Similarly plants with longer spikelets in F_2 must contain fewer factors and a larger number of them should breed pure in F_3. This is actually found to be the case

(Table VI). Hence it may be concluded, that in this cross where the grain length is not modified by shape, the inheritance of grain length is governed by the interaction of three factors, though it is not possible to analyse the extent to which each of these three factors modifies the length.

V. Correlation between length and breadth of grain.

In the collection of more than 400 varieties of rice, it is found that when they are classified according to the length and breadth dimensions, there are very few varieties in the extreme classes.

Table VIII.

Average measurements of length and breadth of types for the year 1925-26.

	Measurements in mm.	Short				Medium				Long				Total
		5·1 to 5·5	5·6 to 6·0	6·1 to 6·5	6·6 to 7·0	7·1 to 7·5	7·6 to 8·0	8·1 to 8·5	8·6 to 9·0	9·1 to 9·5	9·6 to 10·0	10·1 to 10·5	10·6 to 11·0	
Narrow	1·9	..	..	..	..	..	1	..	..	..	..	..	..	1
	2·0	..	1	..	..	2	1	..	..	1	2	..	1	8
	2·1	1	1	..	1	..	2	3	3	..	..	1	..	12
	2·2	1	1	..	..	1	2	4	2	1	1	..	..	13
Medium	2·3	..	1	..	..	..	2	6	1	3	..	..	1	14
	2·4	1	..	..	1	2	4	7	3	4	..	..	..	22
	2·5	..	..	..	..	4	9	12	1	2	1	1	..	30
	2·6	..	..	..	..	4	11	9	4	3	2	..	..	33
	2·7	1	2	..	1	4	7	10	3	2	..	..	..	30
	2·8	..	3	2	1	2	12	10	5	4	..	2	1	42
	2·9	..	3	2	..	8	14	10	12	3	..	1	..	53
	3·0	..	3	3	2	5	19	11	8	1	..	1	..	53
Broad	3·1	..	1	2	..	5	8	12	4	5	1	..	..	38
	3·2	..	..	..	2	1	8	5	6	..	2	..	..	24
	3·3	..	1	..	..	1	2	7	3	..	..	..	..	14
	3·4	..	..	..	..	2	..	..	2	3	..	..	..	7
	3·5	..	..	..	1	..	1	..	3	..	..	..	..	5
	3·7	..	1	..	..	..	..	..	..	..	..	..	..	1
	Total	4	18	9	9	41	103	106	60	32	9	6	3	400

The above suggests the possibility of an association between the length of grain and shape or size. As the thickness of grain in rice varieties does not vary much, the $\frac{\text{length}}{\text{breadth}}$ ratio, together with the length of grain, can be taken as expressive of the shape or size of the grain. This index, $\frac{\text{length}}{\text{breadth}}$, has been employed by Beale [1927] and Sethi [1930] in their attempts at the classification of rice types. When the varieties on the Station are classified and grouped according to the length and $\frac{\text{length}}{\text{breadth}}$ ratio, it is found that there is a definite positive correlation between

these ($r=+0{\cdot}60\pm0{\cdot}18$), indicating that long grains tend to be narrow, and short grains are broader than the long ones.

In the cross dealt with in this paper, the parents, T.55 and T.293, both exhibit the correspondence between length and $\frac{\text{length}}{\text{breadth}}$ ratio, r, the coefficient of correlation, being equivalent to $+0{\cdot}59\pm{\cdot}03$ in one and $+0{\cdot}63\pm{\cdot}03$ in the other. The F_2 progeny of this cross having a greater variability as regards the length of grain, also gives a good positive correlation between length and the index, $r=+0{\cdot}74\pm{\cdot}02$. The same sort of correlation, $r=+0{\cdot}64\pm{\cdot}01$, was also obtained in the F_2 of the cross between a small round grain and the long narrow grain mentioned by Ramiah *et al.*

So far, in one case, where the two parents differed in grain length and the shorter grain was associated with a round shape, the latter proved a simple dominant to the former. In another case described in this paper, where the two parents differed in grain length only, the shape remaining the same, the grain length is found to be controlled by three factors. Further work is in progress to determine the inheritance of the grain size and shape in two other cases, one between T.33 and T.32, where the breadth of the grain alone is the variable, the length remaining constant, and the other between T.33×T.25 where both the length and breadth vary. The analysis of the F_2 results of these two crosses confirm that both the characters, length and breadth, follow a multiple factor type of inheritance (Table IX). Further, in the cross T.33×T.25, where both the attributes, length and breadth, vary, there is a definite correspondence between the length variation and the distribution of $\frac{\text{length}}{\text{breadth}}$ ratio in the F_2s ($r=+{\cdot}639\pm{\cdot}018$). It may be stated, therefore, that the factors controlling length and the factors controlling breadth, though different, are not independent but are interrelated.

TABLE IX.

Parent, F_1 and F_2s of crosses T.33×T.25 and T.33×T.32.

	Mean		Standard Deviation		Coefficient of variation	
	Length mm.	Breadth mm.	Length mm.	Breadth mm.	Length mm.	Breadth mm.
Parents T.33	5·88	2·05	0·135	0·065	2·29	3·17
" T.25	7·14	4·00	0·152	0·124	2·12	3·10
F_1 11965	6·56	2·82	..	..	..	..
F_2 1716	6·67	2·90	0·621	0·220	9·31	7·61
Parents T.33	..	2·05	..	0·065	..	3·17
T.32	..	2·78	..	0·098	..	3·53
F_1 11963	..	2·54	..	..	..	..
F_2 1712	..	2·51	..	0·202	..	8·05

VI. Summary.

1. Among the quantitative characters in rice, size of grain is least affected by environment and hence has been used by various workers in their attempts at classification.

2. For studies on grain size inheritance, a six-grain sample taken from the top or the middle of a panicle is sufficient for a plant.

3. There are indications to show that soil and seasonal conditions affect the grain size and further studies are necessary to assess their relative influences.

4. It had been established previously that the short round type of grain was a simple dominant over the long and narrow type, and it was then surmised that the factor or factors governing the grain length may be different, but might be associated with those controlling grain shape. In a cross between two narrow types, differing only in length, it is found that the inheritance is not simple but is of the multiple-factor type, there being three factors controlling grain length.

5. Definite positive correlations are found between the length of grain and the $\frac{\text{length}}{\text{breadth}}$, an index of the shape, in the parents and in the progenies of crosses.

6. Other crosses which are under study indicate that, like length, the inheritance of the grain width is also of the multiple-factor type.

7. The factors controlling length and those controlling width are not independent but have an interrelationship.

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NITROGEN BALANCE IN BLACK COTTON SOILS IN THE MALWA PLATEAU, I.

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Soon after work on the improvement of cotton-growing in Central India was started at the Institute, it was noticed that applications of *karanja* and safflower cakes in small amounts, supplying 15 lbs. of nitrogen per acre, resulted in a superior growth and final yield [Howard, 1929]. Safflower cake usually gave higher yields. It was found to show similar superiority over other manures when used as a dressing to sugarcane or as a *rab** substitute for rice fields [Mann and Paranjpe, 1918; Knight, 1914].

It was suggested that the safflower cake flocculates the soil and that the improved tilth is responsible for higher yields [Mann, Joshi and Kanitkar, 1912]. The possible effect of improved tilth alone, on cotton yields, was tested under local monsoon conditions, both in the field and in pot-cultures, in 1929 and 1930.

Dressings of superphosphate (at one cwt. per acre) and sulphur (passing through 200 meshes per lin. in., at 20 lbs. per acre) were compared with those of *karanja* and safflower cake (at 6½ mds. per acre). The results were :—

TABLE I.

Manurial experiments on cotton, 1929 and 1930.

Treatment	Total solids in suspension after 48 hrs. (Field soil) grm.	Growth-rate (pot-cultures) per cent. increase		Yield of seed cotton (field-plot)	
		Seedling to growing stage	Growing stage to maturity	1929	1930
				Sr. ch.	Md. sr. ch.
Control	0·122	48·2	250·4	3 7½	2 27 3
Sulphur	0·052	68·3	251·5	3 10½	.. .. ..
Superphosphate	0·058	62·0	232·7	3 13½	.. .. ..
Safflower cake	0·074	94·3	258·0	4 2½	2 39 13
Karanja cake	0·083	109·2	287·1	3 4½	2 34 8

* The burning of cowdung and rubbish on the soil surface before a rice crop.

It appears that the improvement of tilth as indicated by the degree of flocculation, due to sulphur and superphosphate was superior to that due to safflower and *karanja* cakes. The growth-rate was higher, up to the end, for the cakes, while it decreased in later stages with sulphur and superphosphate.

The order of superiority as regards yields in 1929 was: safflower cake, superphosphate, sulphur control, *karanja* cake. In 1930, however, *karanja* gave yields almost equal to safflower cake which was always at the top; this may be due to season. In 1929 the first half of the monsoon was wet and was followed by a dry period, while in 1930 the dry and wet periods were more evenly distributed and were of greater intensity (Fig. 1).

FIG. I.

Fig. 1.—Rainfall in 1929 and 1930.

Taking into account the better tilth produced by sulphur and superphosphate the higher yields due to cakes point to the importance of nitrogen supply. It has been shown that nitrification in black cotton soils with good tilth is superior in the beginning of the rains to that in puddled soils but that this difference falls off later on [Plymen and Bal, 1925]. Cultivation of black soils (though perhaps improving tilth), has been found to have no appreciable effect on nitrification [Annet and Padmanabha Aiyer, 1928]. Not only the amount of nitrogen but the manner in which it becomes available appears to influence yields.

In order to understand clearly the rôle of nitrogen supply in determining cotton yields under local monsoon conditions, it seemed necessary to study closely the nitrification of these two cakes in black soil under conditions resembling those of the field soil in July, August and September—the growing period of the crop. The usual method of incubating thin layers at constant temperatures in flasks or similar containers is not suitable. The variations in humidities and temperatures should be similar to those under field conditions during the period; free aeration should also be provided. The following procedure was therefore adopted.

Experimental method.

Rectangular trays 12 in. × 6 in. and 2 in. deep made of galvanised iron sheet were used as containers. Arrangements were made to divide the soil in the trays into several portions, without disturbing continuity, by inserting wire gauze strips or frames after the trays were filled. Samples could thus be taken at random at every period without disturbing the adjacent soil. Nine trays were placed on a wooden stand 21 in. long, 15 in. broad and 15 in. high with three shelves, so that sufficient interspace for free ventilation was left on all sides of each tray. The wooden stands with trays were kept inside a bottomless humidity chamber 2 ft. 3 in. long, 1 ft. 8 in. broad and 2 ft. 3 in. high. The chamber was made of a wooden frame fitted with glass sides and top and provided with closely-fitting glass doors on both the narrow sides. Two sets of half-inch diameter holes were drilled in the frame, one at the bottom of a narrow side, and the other at the top of the one opposite to it. Hooks were provided to suspend a hygrometer and a thermometer. The humidities were kept under control by keeping shallow trays filled with water or calcium chloride near the bottom ventilators so that incoming air passed over them. The ventilators were always kept at right angles to the prevailing direction of wind to avoid inequalities due to sudden rush of air. The experiment was done under shade in diffused light.

The very coarse material was removed from field soil which was then carefully mixed with the required proportion of manures—equivalent to 17 lbs. of nitrogen per acre in this case—and slowly and uniformly wetted by sprinkled water, keeping the granules intact. The mixture was allowed to rest for one day, and was then

filled into the trays slowly and evenly without packing, right up to the brim. Enough water was then gradually added to bring the moisture within optimum range for nitrification—between 25 and 30 per cent. [Plymen and Bal, 1925]. The moisture was then kept constant within the range by periodically spraying (from a wash-bottle fitted with a rubber bulb) enough water to make up lost weight. The humidities and temperatures in the chamber were recorded and the former maintained within their required limits. Samples were removed from random sections in trays for analysis from time to time. Total nitrogen was determined by the Kjeldahl-Gunning method, combined ammonia by distillation with magnesia, and nitrates by the phenol-disulphonic acid method. Counts of algae were made by the dilution method.

As the added manures do not generally go deep into the soil the depth in the trays was kept at two inches only. In contrast to field conditions, drainage was not allowed and the soil was protected from beating rains and surface wash. These factors, as well as the influence of growing crops, can easily be introduced if necessary under the arrangement used for this test. It is hoped in the future to obtain data by this method, under known conditions approximating to those in the field, which will prove useful as a basis for further field trials.

Experiment I.

In order to get a preliminary idea as to what may happen in different dry and wet monsoon periods, two sets were kept for nitrification, one starting from humidities ranging between 60 per cent. and 70 per cent. and gradually rising to 95 per cent. to 100 per cent. in 12 weeks, and the other maintained at 10 per cent. higher humidity, rising to the same maximum in the same period [Fig. 2].

Treatments with ammonium sulphate (as standard for comparison), *karanja* and safflower cakes were given to the soil. Duplicate samples from set A (lower humidity) were tested for total, ammoniacal and nitrate nitrogen before the start and after 15, 45 and 75 days. Set B (higher humidities) was similarly tested at the start and after 30, 60 and 90 days.

FIG. 2

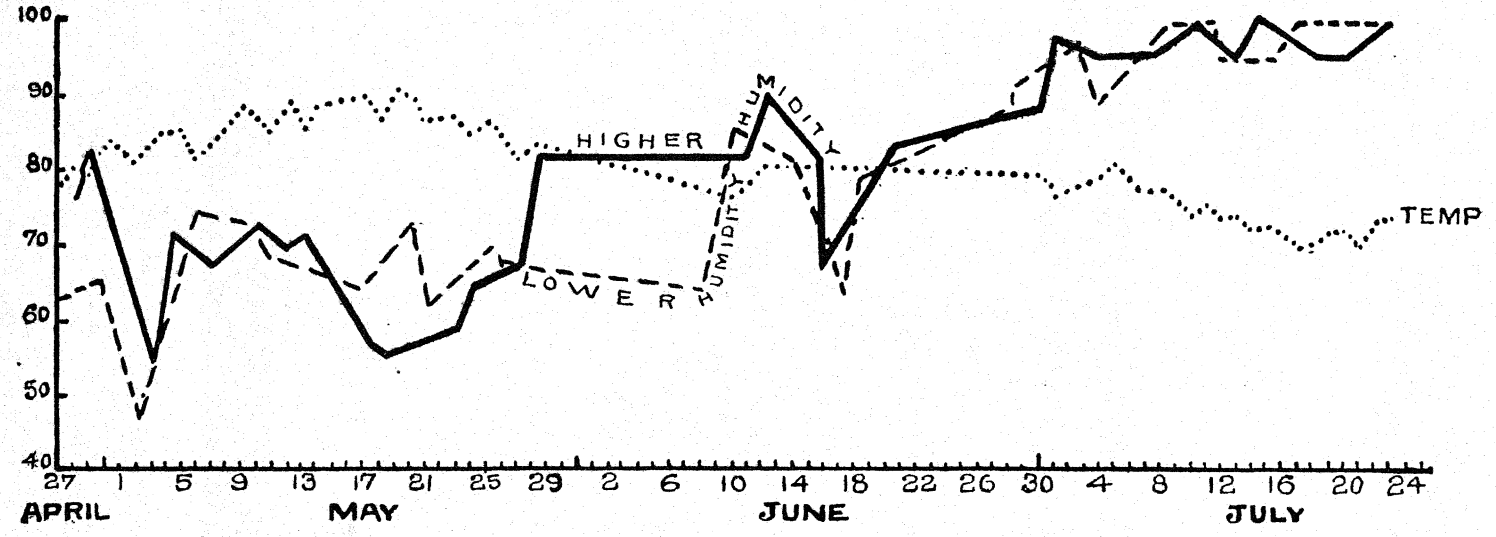

FIG. 3

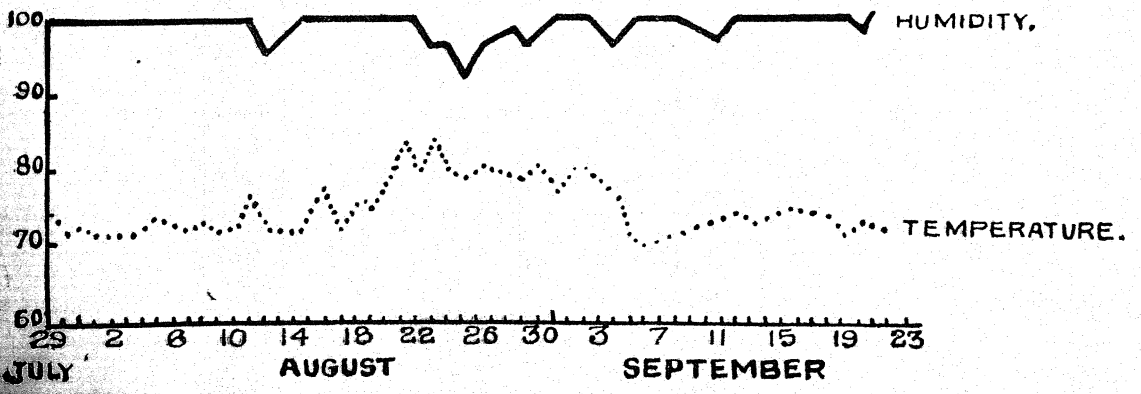

Fig. 2.—Temperatures and humidities in nitrification chambers—Experiment I.

Fig. 3.—Temperatures and humidities in nitrification chambers—Experiment II.

TABLE II.

A

Date of sampling	Period from start	*Karanja* cake					Safflower cake					Ammonium sulphate				
		NH_3—N	NH_3—N per cent. of TN	NO_3—N	NO_3—N per cent. TN	Total N	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N
27th April 1932	At start	..	..	..	..	82·63	..	..	..	..	82·13	..	..	..	..	80·69
11th May 1932	15 days	3·23	3·84	3·41	4·06	84·0	3·11	3·38	2·84	3·08	92·05	2·83	3·61	3·44	4·39	78·4
10th June 1932	45 „	4·13	4·64	2·92	3·28	88·97	3·91	4·33	2·68	2·97	90·21	4·21	4·87	2·80	3·24	86·37
11th July 1932	75 „	..	..	3·12	3·48	89·7	..	..	3·03	3·39	89·32	..	..	3·35	3·72	90·0

B

Date of sampling	Period from start	*Karanja* cake					Safflower cake					Ammonium sulphate				
		NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N
27th April 1932	At start	..	..	..	..	82·63	..	..	..	..	82·13	..	..	..	..	80·69
26th May 1932	30 days	6·33	7·79	2·23	2·75	81·2	6·36	7·92	2·58	3·21	80·31	6·28	7·68	2·20	2·69	81·74
25th June 1932	60 „	5·18	6·43	2·03	2·52	80·5	5·20	6·28	2·15	2·60	82·81	5·03	5·60	2·30	2·56	89·84
28th July 1932	90 „	2·32	2·80	1·61	1·94	82·7	2·10	2·48	1·96	2·31	84·70	1·83	2·14	2·07	2·42	85·25

Experiment 1. A, Lower humidity ; B, Higher humidity in mgm. per hundred grms. of soil (oven-dry).

Algal growths appeared after about a month and were found to be mainly *vaucharia*. General observations on the intensity of the growth were recorded and total counts per gramme of wet soil as well as the vertical distribution in the soil depth were estimated at the end of the experiment—twelve weeks.

TABLE III.

A, Intensities of algal growths ; B, total counts and distribution in soil depths.

A

Treatment	1½ months	2 months	2½ months	3 months
Karanja cake	No growth	Most vigorous	Less vigorous	Almost unchanged
Safflower cake	No growth	Most vigorous	Vigorous	Vigorous
Ammonium sulphate	A little growth	Most vigorous	Much less vigorous	Still less vigorous

B

Treatment	Algae per gm. of soil				Total
	0 to ¼-in.	¼ to ½-in.	½ to ¾-in.	¾ to 1-in.	
Karanja cake	5,000	4,400	600	*nil*	10,000
Safflower cake	12,000	7,000	3,750	,,	22,750
Ammonium sulphate	8,000	3,750	1,850	,,	13,600

Experiment II.

A second set including the same manurial treatment was then started, keeping the humidity between 95 per cent. and 100 per cent. (Fig. 3).

Duplicate samples were withdrawn every week for analysis. The results obtained in eight weeks, are given below :—

TABLE IV.

Mgm. nitrogen per 100 grms. soil (oven-dry).

Date of sampling 1932	Period from start	*Karanja* cake					Safflower cake					Ammonium sulphate				
		NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N	NH_3—N	NH_3—N per cent. TN	NO_3—N	NO_3—N per cent. TN	Total N
29th July	At Start	..	..	..	..	73·81	..	..	..	..	72·58	..	..	..	..	72·09
4th August	1 week	4·29	5·64	4·12	5·42	76·08	5·42	7·36	4·40	5·97	73·66	5·80	7·98	3·89	5·35	72·68
11th ,,	2 weeks	4·17	5·98	4·59	6·58	69·75	4·07	5·38	4·15	5·48	75·69	4·14	5·67	4·53	6·20	73·03
18th ,,	3 ,,	2·29	3·03	2·83	3·75	75·52	2·30	5·48	2·88	4·35	66·14	2·30	3·15	3·36	4·60	73·07
25th ,,	4 ,,	1·53	2·07	2·51	3·39	73·86	1·57	2·08	2·62	3·47	75·56	1·51	2·05	2·59	3·52	73·57
1st September	5 ,,	1·53	2·01	2·78	3·65	76·16	1·56	2·02	2·88	3·73	77·17	1·56	2·10	2·91	3·93	74·10
8th ,,	6 ,,	4·28	5·59	1·50	1·96	76·54	4·32	5·72	1·51	2·00	75·46	4·17	5·57	1·51	2·02	74·84
15th ,,	7 ,,	3·53	4·60	1·19	1·55	76·70	3·57	4·67	1·09	1·42	76·47	3·54	4·65	1·11	1·46	76·14
22nd ,,	8 ,,	3·09	4·23	2·60	3·59	72·52	3·13	4·47	2·58	3·69	69·97	3·09	4·19	2·55	3·45	73·80

Algæ appeared after three weeks. Total counts were made every week after, on three-quarters of an inch depth only, because no algæ had been found below that depth.

TABLE V.

Experiment II. Algal counts in a layer three-quarters of an inch deep.

Treatment	Period from start				
	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks
Karanja cake	*nil*	1550	2800	925	300
Safflower cake	300	625	300	900	925
Ammonium sulphate	925	3675	3425	1875	925

DISCUSSION OF THE RESULTS AND CONCLUSIONS.

It will be noticed that under conditions of greater humidity ammoniacal nitrogen is higher, and nitrate nitrogen lower than in the drier atmosphere. Under drier conditions *karanja* and ammonium sulphate accumulate more nitrates than safflower cake. In a wetter atmosphere, cakes accumulate nitrates more rapidly to begin with, but are soon overtaken by ammonium sulphate. With all treatments nitrates rapidly fall in the third week, are lowest for *karanja* and remain so afterwards.

The nitrates from ammonium sulphate remain slightly higher than from safflower cake. This shows the variation in nitrates available to plants during these periods. The appearance of algal growth is simultaneous with the fall in nitrates. There is no loss of total nitrogen in any case.

One point seems to be worth notice, *viz.*, that the actual quantities of nitrates found in the absence of drainage are far lower than those obtained in black cotton soil by other workers [Plymen and Bal; 1919, 1922]. Similar low content had been observed when nitrification of composts was tested [Howard and Wad, 1931]. These low figures in the absence of drainage losses are of special interest, especially in view of the quick and vigorous crop-growth usually found in the field. An examination of algal counts indicates that whenever nitrate accumulations reach a certain limit, they are utilised by algæ, leaving a small amount available to plants. Under cropped conditions the excess over the minimum will be shared between plants and algæ, the exact division being determined by the comparatively favourable or unfavourable nature of the environment for either. The actual

quantities of nitrates produced are the sum of those found in the free state and those absorbed by algæ, which gives the real guide for estimation of the nitrate-producing capacities of different manures. The superiority of safflower cake, both as to total and steady nitrification is thus very clear. Unlike *karanja* cake and ammonium sulphate, which exhaust themselves sooner, safflower cake is capable of nourishing plants right up to the end and gives higher yields. Its comparative slowness in the early period does not handicap the cotton plant .whose requirements are small at the time. Prescott's observation [1918] that nitrates accumulate in soil under cotton in early stages while they do not doso under wheat and maize is pertinent.

The algæ seem to serve as traps for excess nitrates under humid conditions when they are likely to be lost by leaching or otherwise. The degree of leaching of nitrates from soil as well as the extent of algal growth varies with its wetness and they may thus automatically balance each other. Possible loss of nitrates by leaching is thus confined to a small fraction of the nitrates actually present in the soil after absorption by plants and algæ. This algal factor appears to operate in this way in Malwa during the monsoon, for in 1932, after a wet period of 9·35 inches of rain in five days, ending on September 9th, profuse algal growth, mainly *vaucharia*, was discovered in several fields. The nitrates thus trapped by algæ can obviously be returned for plant absorption later on. The presence of algæ in arable soils has been shown [Bristol-Roach, 1927]. Their rôle in nitrogen conservation and in reducing leaching has been mentioned by Russell and Richards [1920], Russell [1923], Waksman [1929], Prescott and Piper [1930], and Howard and Wad [1931].

General yellowing of seedling crops during continued spells of wet weather is a frequent phenomenon on the Malwa plateau. The plants quickly recover after the rains stop. Attempts to trace denitrification in the flooded fields proved futile. Repeated addition of nitrites to pot-grown plants flooded even up to leaf-fall also did not result in yellowing. The absence of denitrification was further proved by another observation in the unusually long wet period (45 inches during a continuous period of 42 days) in 1932, when all seedling crops yellowed and became stunted. Ratoons of the previous year as well as crops sown in May remained quite unaffected and dark green. They continued to grow with normal vigour, unchecked, and by the beginning of October were full-grown plants three or four times larger than the June-sown stunted crop. The starvation of seedling crops side by side with adequate nourishment of older plants indicates a poverty of nitrates only a few inches deep, in soil around the active zone of the younger plants. Leaching by surface-wash, competition from absorbing micro-organisms or temporary suspension of nitrification due to excessive moisture seem to be responsible, singly or jointly. The presence of algæ also precludes the possibility of denitrification. Russell [1914]

saw no possibility of denitrification in arable soil. Subrahmanyan [1927] has shown that there is no loss of total or volatile nitrogen and that no bacterial denitrification takes place in water-logged soils. Nitrification stops only temporarily, to be revived at a rapid rate on drying. It will be of interest to determine whether, under field conditions, rapid recovery of crops on drying is mainly due to rise of nitrates from lower layers or to quick revival of nitrifying activity. Denitrification where the crop never recovers, may occur in small isolated patches in fields and the crop may remain diseased and stunted or may be completely wiped out. This has no economic significance [Waksman, 1931].

It is possible that accurate information on conditions that control nitrogen-balance in manured and unmanured soils will permit of a more efficient management of crop-nutrition even under the reputedly uncontrollable monsoon conditions. The results with safflower cake show that a steady nitrogen supply and storage of the surplus, as reserve, in a stable yet easily available form, are likely factors for securing high yields on crops exposed to uncertain environment. It should be possible to use the reabsorption factor by suitable agronomic adjustments to control the transformation of soil organic matter, original or added, so as to regulate the supply of nutrients according to the needs and capacity of growing plants at a given period. Wastage being thus avoided, introductions of intensive crops will be facilitated. A fresh avenue appears to be opened for work along these lines, which may quickly yield results of practical value.

Summary.

(1) Higher yields of cotton from dressings of safflower cake have been shown to be due, not so much to improvement in soil tilth, but to appropriate supply of nitrogen.

(2) A suitable method of studying nitrification in soil has been described.

(3) In black cotton soil the concentration of free nitrates has been found to be very low under the conditions of the experiment.

(4) Algæ have been shown to be an important factor in conservation of nitrates.

(5) The possibilities of the absence of appreciable leaching and denitrification in arable soils has been discussed.

Acknowledgment.

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MEKRAN—POSSIBLY THE COUNTRY OF ORIGIN OF THE GREAT LOCUST INVASION OF SIND IN 1926.

BY

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(With Plates LXV and LXVI and two maps.)

INTRODUCTION.

The last great outbreak of locusts in India had occurred in the provinces of the Punjab, Sind, Baluchistan and Rajputana during the years 1913, 1914 and 1915, and since 1920 hardly anywhere had locusts been noticed in India. People had, indeed, well nigh lulled themselves into the belief that locust infestation had become a thing of the past, when, all of a sudden, large swarms of them appeared in August-September 1926, in Sind, and bred extensively in that province. Later in the same year, enormous swarms of pink locusts were found flying all over Sind, spreading into the states of Kathiawar and North Gujarat on the south, and entering the southern districts of the Punjab on the north. Early in the season, during the year following (1927), egg-laying occurred in various districts in the Punjab and the United Provinces in March and the North-West Frontier Province in April; and also in different parts of Baluchistan during the spring months. With this, a recurrent annual infestation, that was at its worst during 1929, started over the whole of an area, extending from Baluchistan on the west to the United Provinces and Central India on the east, and lasted till November 1931.

The origin of this infestation has, however, remained more or less a mystery for want of records of observations prior to September 1926 in regard to the entrance of the initial swarms. An excellent account of the infestation of 1926-27 in Sind and Kathiawar is given by Mann and Burns [1927]. They state as follows: "In point of time, the earliest notice we have of the attack under discussion is the appearance of locusts on the 25th September 1926 at Sujawal, Jati and Shahbunder in Karachi District. The Deputy Collector, Tatta (Karachi district) reported on November 3rd to the Collector of Karachi that locusts still in the hopper stage had been noticed all round the Karachi taluka and that they were in abundance in the *dehs* near the Habb River. On the 16th November, the Deputy Director

of Agriculture in Sind reported that locusts had developed wings and become scattered. This is the only available report describing any locusts actually in the hopper stage ".

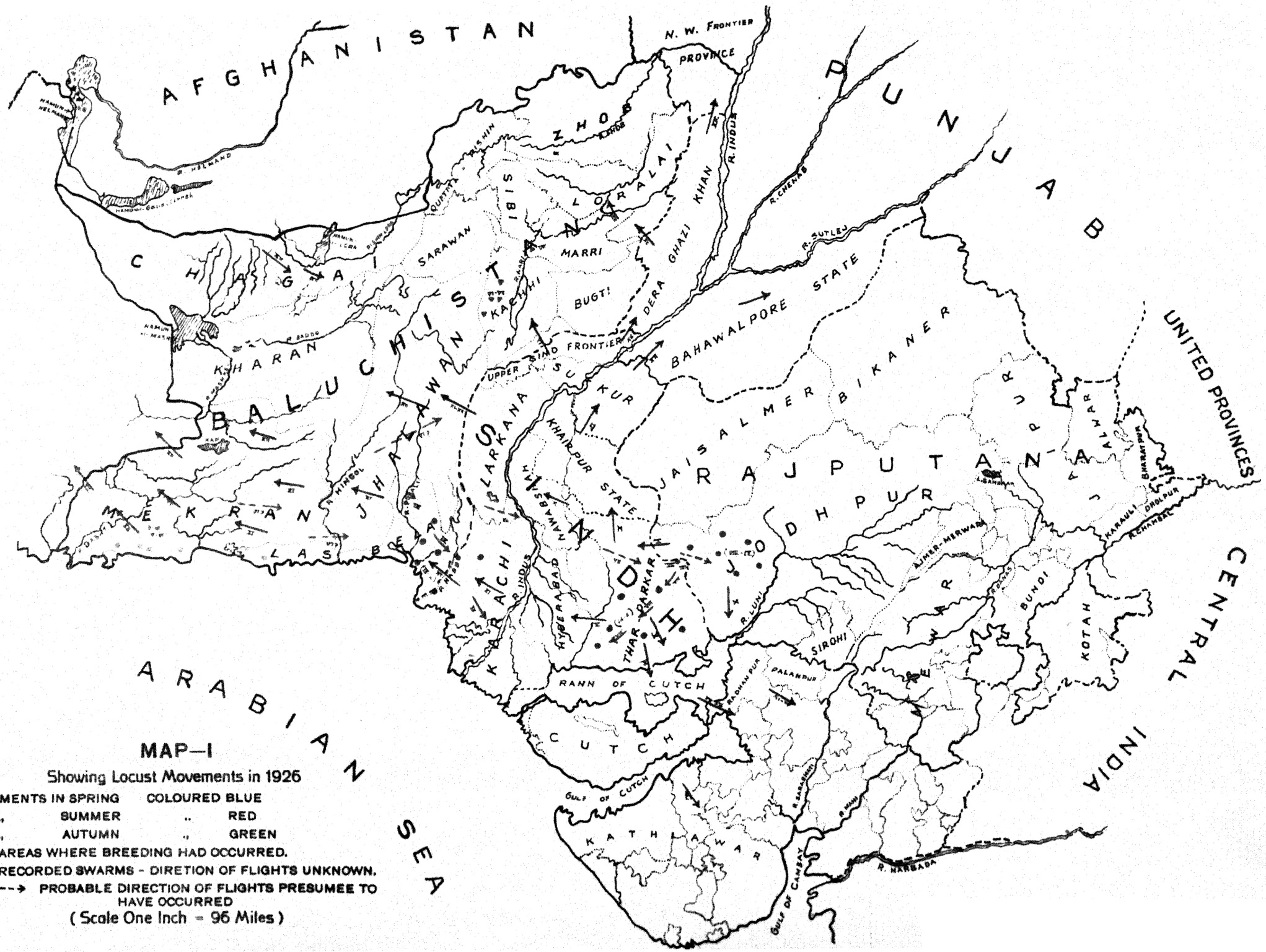
Referring to the origin of the infestation of 1926, they remark : " The only observed place of origin of these locusts was the Habb River-bed near Karachi, but the desert areas of Thar and Parkar is also a possible source ".

One of the objects of the scheme of Locust Research inaugurated by the Imperial Council of Agricultural Research is the location of the permanent breeding areas of the desert locust within the limits of British India, and in the course of the locust survey tours undertaken for this purpose, locusts of the solitary phase have been found in some of the areas examined, chiefly :—(1) on the Mekran Coast in South Baluchistan ; (2) in the Indus Valley in Sind and South Punjab ; and (3) in parts of the Great Indian Desert. The relative importance of these areas as real " reserves " [Uvarov, 1932] of the locust can, however, be determined only by continuous observations made over a series of seasons during a non-locust cycle. In the opinion of the writer, however, it may also be possible to elucidate this by an intensive study of the great locust invasions of the past, whereby a clue may possibly be obtained as to which of these areas had functioned in individual cases as starters of the swarms.

Locust swarms do not usually attract any attention until the outbreak has attained serious dimensions, and thus the initial swarms, which alone are of importance for getting clues as to their origin, are naturally never noticed or recorded. Again, old records on locusts have proved to be a sort of *rara avis* in most of the offices, since they have, as a rule, not been able to escape the periodical destructive activities of the Record-Keeper in his endeavour to keep the prolific growth of files under his care within reasonable limits. It is only by a fortuitous and fortunate set of circumstances that a few old records of value have actually been found preserved in some places. The material thus discovered has been perused and notes taken with the object of making a comprehensive scrutiny of the whole question. Particular attention was paid to the records of the period immediately preceding the attack of 1926, with the object of tracing out the origin of the last great infestation.

EXTENT OF BREEDING IN 1926.

In the course of the survey tours in Baluchistan, Sind and Rajputana, special attention was paid to this question, by collecting all available records on the subject, and making enquiries of responsible persons as to their remembrance of events in the past, especially in regard to 1926, with the result that considerable enlightenment has been obtained on the subject (Map I).



MAP—I

Showing Locust Movements in 1926

MOVEMENTS IN SPRING COLOURED BLUE
" SUMMER " RED
" AUTUMN " GREEN
● AREAS WHERE BREEDING HAD OCCURRED.
▲ RECORDED SWARMS - DIRETION OF FLIGHTS UNKNOWN.
----→ PROBABLE DIRECTION OF FLIGHTS PRESUMEE TO HAVE OCCURRED

(Scale One Inch = 96 Miles)

In Lasbela, the earliest record for 1926 is that of an occurrence of swarms of locusts on 24th August, at Sheh Lakhra attacking germinating crops, but without any indication as to whence they had arrived. In September, eggs were reported to have been laid in various places: *e.g.*, the *ilaquas* of Uthal, Sonmiani and Habnadi, and in October-November, hoppers were stated to have appeared therefrom. In November-December, large swarms of pink locusts appeared all over the State coming from an eastern direction.

In the Thar-Parkar district of Sind, the earliest mention of locusts is in a report regarding an emergence of hoppers, towards the close of August 1926, at Gadra in taluka Chachhro, on the borders of the Sheo Pargana in Jodhpur State. Towards the end of August, flights of yellow locusts are recorded as having passed south from Chachhro into the desert portion of Nagar-Parkar taluka, and westwards into Mithi and Diplo talukas, and laid eggs therein. In Umarkot taluka also locusts were reported to have appeared from the east, and laid eggs all over the desert portions in September; and huge numbers of hoppers were noted in October. In October-November 1926, large flights of pink locusts appeared all over the district, passing from east to west, and causing much damage to crops.

In the Mallani area of Jodhpur State, adjoining the Thar-Parkar district, swarms were said to have appeared, in the month of *Savon* of Samwat 1983 (*i.e.*, August, 1926) from the west, and to have led to extensive breeding in the desert portions of Western Marwar.

There is thus clear evidence to show that, besides the Habb River tract recorded by Mann and Burns, there were in addition vast areas of breeding in the Lasbela State, in the Thar-Parkar deserts, and in the Mallani and Sheo Parganas of Jodhpur State in September-October 1926; and this would serve to explain the sources from which the immense swarms of locusts, that appeared in the autumn of 1926 and spread over the Punjab, Sind, United Provinces, Rajputana, Kathiawar and North Gujarat, had been derived.

In Mekran, records indicate that in May-June 1926, there were large swarms of hoppers and flying locusts in Kulanch. Fliers continued to be present there till the middle of July, after which they are said to have "disappeared". It is also on record that between October and December 1926, large swarms of pink locusts had appeared from the east in the Niabats of Turbat, Kolwah, Dasht and Panjgur, and passed on towards the west.

In Kachhi Division of Kalat State, there are records of the damage caused by the appearance of locusts in various villages of Mirpur-Nasirabad Niabat (*i.e.*, round about Gandhawa) between the 25th June and 10th July, 1926. At about the same time, locusts were reported to have destroyed the cotton crops at Kurk village, in the adjoining British area in Sibi district (1st week of July, 1926).

In Jhalawan (Kalat State), locusts are recorded to have been present in very large numbers in Khuzdar Niabat, during December, 1926 (presumably entrants from Sind).

Direction of locust movements in Baluchistan.

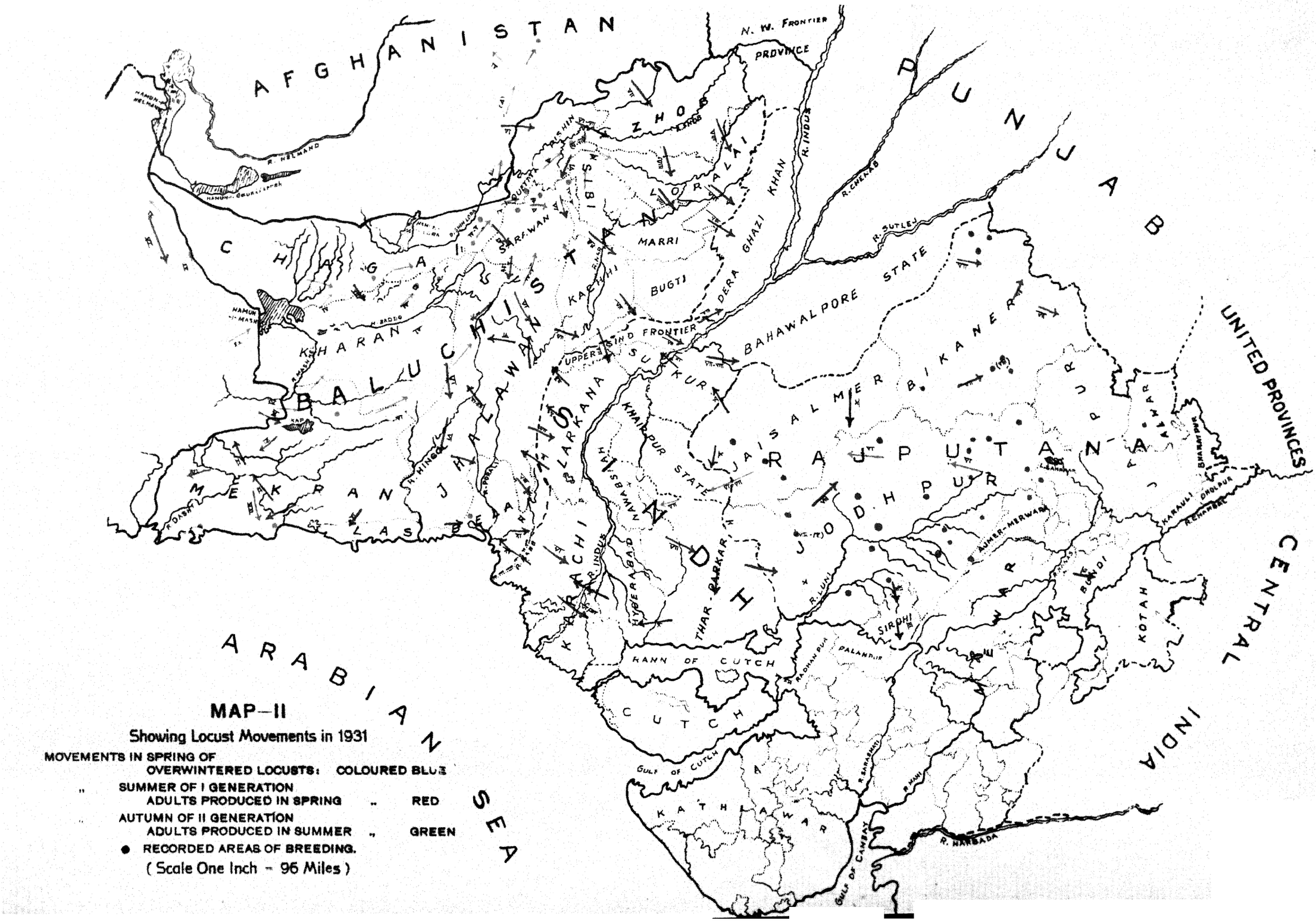
During the year 1931, the writer had the opportunity of studying the direction of locust flights in Baluchistan as reports came in, and the following general movements were noted (Map II), which indicate that there is a certain regularity in the direction of their movements during the different seasons of the year :—

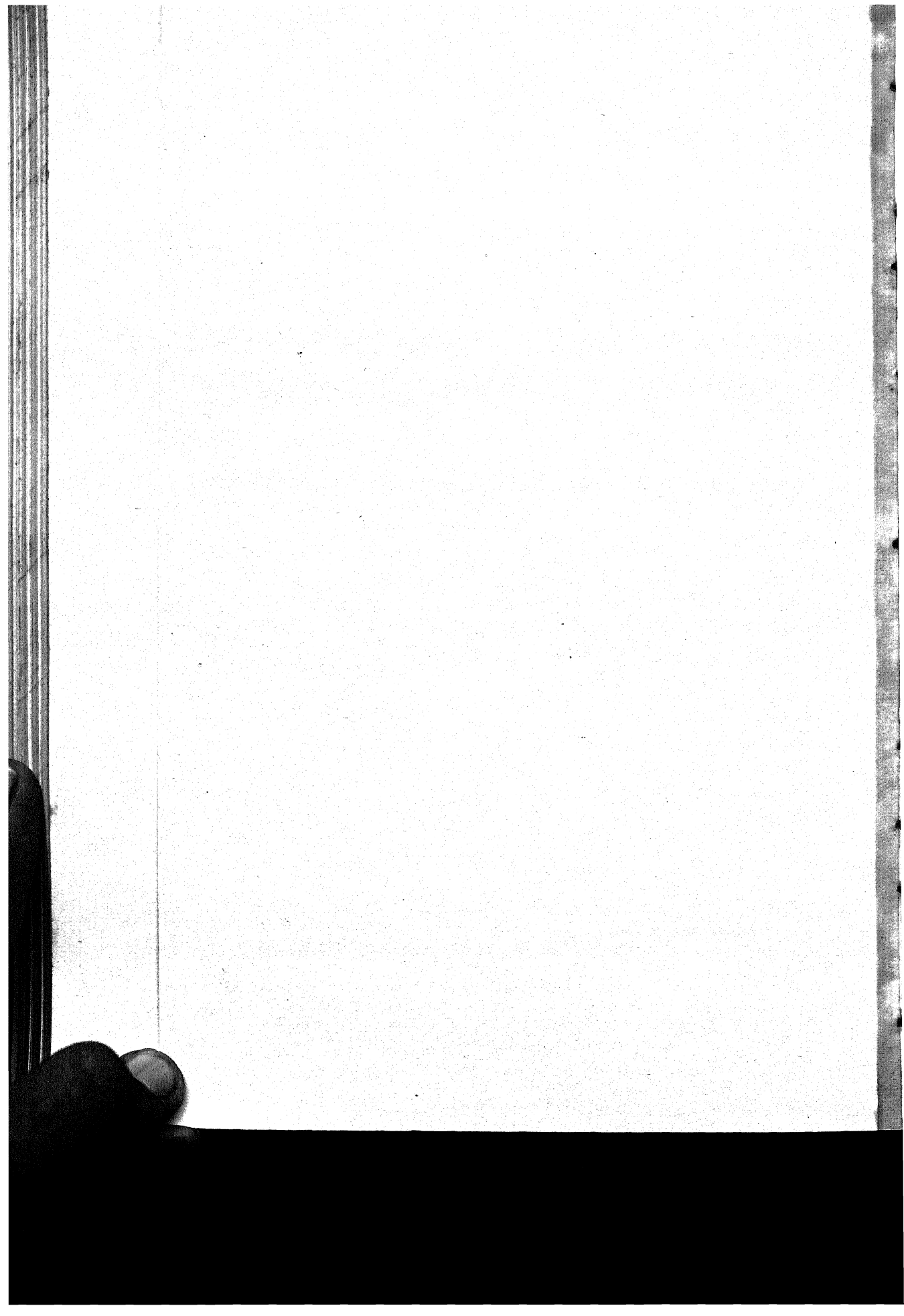
I. During the spring months, flights of over-wintered yellow locusts started :— (1) from the Persian borders on the west into Kharan, Chagai, Quetta-Pishin, Sarawan, etc. ; (2) from the Persian boundary on the south-west into Mekran, thence into Jhalawan and Sarawan ; and (3) from Sind and Kachhi on the east, westwards into Sarawan, and north-eastwards into the Bolan and Harnai areas. These migrations resulted in egg laying in suitable places, and the subsequent emergence of hoppers.

II. In summer, the pink locusts produced from these hoppers were found flying during the months of May, June, July and August, in a general eastern direction :— (*a*) from the direction of Afghanistan, *via* Quetta-Pishin, Zhob and Loralai, into the Punjab ; (*b*) from Chagai, *via* Sarawan, Quetta-Pishin, Bolan and Kachhi, or *via* Sarawan, Jhalawan and Kachhi, into Sind ; and (*c*) from Mekran into Jhalawan and Lasbela, and ultimately into Sind. A scrutiny of the movements reported in Sind and Rajputana during this part of the year shows that part at least of these swarms passed on, in an eastern direction over Sind into Rajputana in August-September, and laid eggs there.

III. In autumn, *i.e.*, between September and December, reports received from Lasbela showed an immigration of pink swarms from Sind ; other reports from Mekran were to the effect that several pink flights hailing from the east had passed on towards the north-west ; and still others from Jhalawan indicated that certain pink swarms from an eastern source were passing westwards into Kharan. At the same time, there were reports of flights from Sind into Kachhi. Reports of the same period as to flights in Sind and Rajputana also indicated a similar westward trend in their movements.

All available records for the years 1926, 1927, 1928, 1929 and 1930 in Baluchistan and Sind have been examined and these also show clearly that the general trend in locust movements during the spring, summer and autumn periods is, with slight variations, almost similar to that of 1931, the general direction being :—(1) towards east and north-east in spring ; (2) towards east and south-east in summer ; and (3) towards the west, south-west and north-west in autumn.


AFGHANISTAN
N. W. FRONTIER PROVINCE
PUNJAB
BAHAWALPORE STATE
UNITED PROVINCES
CENTRAL INDIA
RAJPUTANA
JODHPUR
JAISALMER
BIKANER
CHAGAI
BALUCHISTAN
KHARAN
MEKRAN
LASBELA
JHALAWAN
SARAWAN
KALAT
QUETTA
ZHOB
LORALAI
MARRI
BUGTI
KACHHI
KHAIRPUR STATE
THAR-PARKAR
KUTCH
KATHIAWAR
ARABIAN SEA
Gulf of Cutch
Gulf of Cambay
R. HELMAND
R. INDUS
R. SUTLEJ
R. NARBADA
MAP—II
Showing Locust Movements in 1931
MOVEMENTS IN SPRING OF
OVERWINTERED LOCUSTS: COLOURED BLUE
SUMMER OF I GENERATION
ADULTS PRODUCED IN SPRING .. RED
AUTUMN OF II GENERATION
ADULTS PRODUCED IN SUMMER .. GREEN
RECORDED AREAS OF BREEDING.
(Scale One Inch - 96 Miles)



There also appears to be little doubt that a fair proportion of the western flights had usually reached Persia almost every year in winter, and over-wintered there*. In spring, many of these swarms would appear (possibly in conjunction with locusts of Persian or Arabian origin) to have turned eastwards again into Mekran and Chagai, thus recommencing the circuit. It is possibly due to this seasonal circulatory movement—presumably directed towards regions of likely rainfall, *i.e.*, in autumn and spring towards areas of winter precipitation, and in summer towards regions of monsoon rainfall,—that the infestation, once it starts, continues for a series of years.

Origin of the 1926 swarms.

The breeding areas of the summer season of 1926 in India would appear to be divisible into the following two distinct groups:—(1) a western one—inclusive of the areas of Lasbela and Karachi; and (2) an eastern one—comprising the desert tracts of Thar-Parkar district and Mallani. In the second group, breeding would appear to have started much earlier than in the first,—a fact which appears to be correlated with an earlier receipt of rainfall. In Thar-Parkar and West Rajputana good rains fell in July and August, and continued into September, whereas in Lasbela and Karachi, rains commenced only in August, though, later on, very heavy rains were received in September as the result of a storm that originated in Gujarat, and moved on over South Rajputana towards the Sind Coast, early in that month.

So far as the western area is concerned, it appears to be reasonable to suppose that the origin of the infestation was the swarms of June-July, 1926, recorded in Kulanch. Since the general direction of flight of locust swarms produced in summer in the Mekran area is to the east, towards Lasbela and Sind, the fliers noted at Sheh Lakhra on the 24th August 1926 were doubtless of Kulanch origin. By September, the swarms had spread over the whole of Lasbela and Karachi and reached as far east as Jati, Sujawal, and Shah Bunder (25th September). At the same time, widespread oviposition had followed the heavy rains received in many parts of Lasbela and Karachi.

* In this connection, it may be mentioned that the following writers state that the Persian infestation of 1927 had actually been derived in great part from an Indian origin:—Fletcher [1929] states "The eastern boundaries of Persia were heavily invaded in 1927 by swarms said to have come from Sind and Baluchistan." Noskov [1928]: "Swarms of *Schistocerca gregaria* Forsk. migrated from India into Persia in 1927, and an enormous area in the east, stretching from the sea almost to the northern frontier, was infested with eggs. In the northern districts, the larvae hatched about the middle of May and reached the adult stage early in July." Moritz [1928]: "The swarms of *Schistocerca gregaria*, which came from India and Southern Afghanistan, invaded a vast area, and the eggs laid by them hatched in May-June. New flying swarms moved mainly in an easterly or north-easterly direction, and many of them apparently penetrated into Afghanistan".

As to the eastern area, *viz.*, Thar-Parkar and Mallani, it is not clear wherefrom the initial swarms had originated. A study of the movements of locusts in Sind in summer, indicates that a large proportion of the swarms come, during May, June and July, from the direction of Baluchistan, pass over the Sehwan and Dadu areas into Nawabshah district, and thence move eastwards into the desert portions of Khipro, Umarkot and Chachhro talukas, and ultimately into the Sheo and Mallani deserts of Marwar. It is not inconceivable that the initial swarms of 1926 had arrived from Mekran side by this route and in view of the early rainfall received in July in the Chachhro and Mallani tracts, had laid eggs earlier than the western swarms. If this view be correct, it is evident that the eastern infestation may have been derived from the same source as the western.

On the other hand, small swarms of locusts are recorded to have occurred in the Dera Ghazi Khan district (Punjab) along the west bank of the Indus in 1922 and 1925 [Afzal Husain, 1929], and it is just possible that small colonies of the solitary phase locust had been existent all along the Indus Valley during the period 1920-25, and had developed into big swarms by rapid multiplication, induced by the good and widely distributed spring rainfall recorded all over Sind and Rajputana in the months of January, March, and May, 1926 (Table I). That such breeding is possible is shown by the observations made as to the occurrence of sporadic breeding in April-May, 1932, at Angare-Gadap in Karachi taluka, and at Thari in Khairpore State, but further continued observations on this subject are needed before any definite opinion can be pronounced on this view of the origin of the eastern infestation of 1926.

TABLE I.

Monthly rainfall data in inches for the year 1926.

Localities	Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Baluchistan :—													
Panjgur	3·69	0·04	1·38	0·05	0·62	0·01	..	0·51	0·65	..	..	1·15	8·10
Turbat	7·83	0·31	1·84	..	0·49	..	1·73	0·07	..	..	..	1·17	13·44
Pasni	7·54	0·04	0·48	..	..	..	0·02	0·32	0·09	..	..	..	8·49
Ormara	13·80	..	..	..	..	..	..	1·34	0·45	..	..	..	15·59
Sonmiani	0·16	..	0·50	..	..	..	..	0·40	5·30	..	..	..	6·36
Bela	1·87	..	0·63	..	2·07	0·54	0·58	0·55	2·79	..	..	..	9·03

TABLE I—*contd.*

Localities	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Total
Sind :—													
Karachi .	0·28	..	0·16	..	..	..	0·78	3·47	15·35	..	..	..	20·04
Hyderabad	0·04	..	1·65	..	0·45	..	3·09	5·08	1·79	..	..	..	12·10
Jacobabad	0·35	..	0·90	..	0·94	..	0·93	1·09	0·06	..	..	..	4·27
Diplo	0·12	..	0·23	..	0·77	..	3·96	2·88	12·64	..	..	0·40	21·00
Chachhro	0·66	..	0·04	..	2·13	..	2·25	7·91	5·52	..	..	..	18·51
Mithi	1·00	..	0·80	..	1·61	..	9·44	6·73	10·06	..	..	..	29·64
Nagar-Parkar	..	..	..	..	0·22	0·05	3·71	13·55	16·07	..	..	..	33·60
Jamesabad	0·30	..	1·37	..	1·28	..	1·83	9·09	2·33	..	..	..	16·20
Umerkot	0·64	..	0·38	..	1·75	..	2·40	6·12	1·53	..	..	..	12·82
Rajputana :—													
Jodhpur	0·72	..	1·80	0·16	0·48	0·17	3·66	7·25	7·20	..	..	..	21·44
Bikaner	0·05	..	0·36	..	0·90	..	4·85	4·29	1·16	..	..	0·21	11·82
Barmer .	0·32	..	0·90	..	1·11	..	3·51	4·17	8·35	..	..	..	18·36
Sheo	0·56	..	1·38	..	0·20	..	3·65	1·39	2·20	..	..	..	9·38
Pachpadra	0·93	..	0·95	..	2·80	..	4·28	2·65	7·29	..	..	..	18·90
Jaisalmer	0·15	..	0·02	..	1·32	..	2·52	6·25	2·30	..	..	..	12·56

PROBABLE ORIGIN OF THE KULANCH SWARMS.

While in most parts of India and Baluchistan, there was no report of locust infestation between the years 1920 and 1925, Mekran records show that there was an outbreak of locusts though of limited extent—in the Niabat of Dasht, not far from the Mekran Coastal area in May-June 1923. Hoppers are said to have been found in millions at Zarrain Bug and Hasadi, damaging *sohru* (red sorghum), cotton and pulses. They acquired wings by the middle of June, and after flying about locally are said to have disappeared into the jungles by the end of June. Information was obtained from Choudhry Khair Muhammad—an Officer of Lasbela State who happened to be Muhasib at Ormara between 1921 and 1927, to the effect that locust swarms had appeared at Ormara in the summer of 1923 from an eastern direction. Nothing is, however, known of the subsequent history of the summer swarms of Dasht and Ormara.

In Persia, an invasion of flying locusts is recorded at Kermanshah during 1923, but presumably this was from an entirely different source.

After 1923, there is no mention of locusts in the Mekran records, till June 1926, when large swarms of fliers are recorded to have been present in Kulanch attacking cotton and pulses, as also large numbers of hoppers. The following information was obtained by personal enquiry from Sayid Imdad Husain Shah

who was Naib of Pasni from 1925 to 1927. In January 1926, there had been heavy rains at Pasni, and indeed, all over Kulanch and the Mekran Coast. About March-April, large numbers of hoppers had been noticed in various places in Kulanch: Nokbur, Gano, Kandiri, Nalent, Kandasole and Kappar. Subsequently large swarms of flying locusts were seen in Kulanch. Large flying swarms were also seen in the Pasni Niabat in October-November, 1926. In the spring months of 1927, locust hoppers were found in swarms all over the Pasni Niabat, especially along the bed of the Saur Kaur, and measures for destruction were taken.

Choudhry Khair Muhammad who had been Muhasib at Ormara in 1926, stated that he had clear remembrance of the extraordinarily heavy rains (13·80 inches) that fell at Ormara in January 1926, and also of certain swarms that had appeared from Pasni side in March-April, and laid eggs in the "reks".

With reference to the possibility of the infestations of 1923 and 1926 having been the resultants of incursions of foreign swarms, there is nothing in the records to indicate the appearance of any at these periods, but on account of the general scantiness of information in the available records such a contingency cannot, of course, be entirely precluded. The locust cycle of 1926-1931 appears to have come into existence more or less simultaneously in parts of Africa and Asia. In the Africo-Arabian area, the earliest swarms were noted by Johnston on the Red Sea Coastal plains of Sudan in the winter of 1925-26, while it was only in 1927 that invasions were reported in Sudan, Egypt, and Morocco. In the case of Somaliland, Kenya, and Tanganyika, as well as of Syria, Palestine, and Iraq, the first swarms were noted only in 1928. In the Indo-Persian area, the first swarms were recorded in Mekran and Sind in 1926, while invasions were reported from Persia, Afghanistan, the Punjab, and United Provinces only in 1927, and from Central Asia only in 1928-29. This probably indicates that there were two or more independent centres of development in Africa and Asia, and that infestations had originated presumably by an overmultiplication of solitary phase locusts in different reserves, leading to the formation of swarms that, later on, spread into the surrounding countries in the course of one or two years.

"Reks" of the Mekran coast.

A survey of the Mekran coast, made during the years 1931-32, has disclosed the existence of certain remarkable breeding grounds of the desert locust, known locally as "reks", extending along the Coast of Mekran from Naka Kharrari and Sonmiani on the east to Pishukan and Jiwani on the west. It is extremely likely that such "reks" are also to be found in the coastal region of the neighbouring area of Persian Mekran, and possibly also on the sea-board of the Persian Gulf—both on the Persian and Arabian sides. "Rek" is the name given to

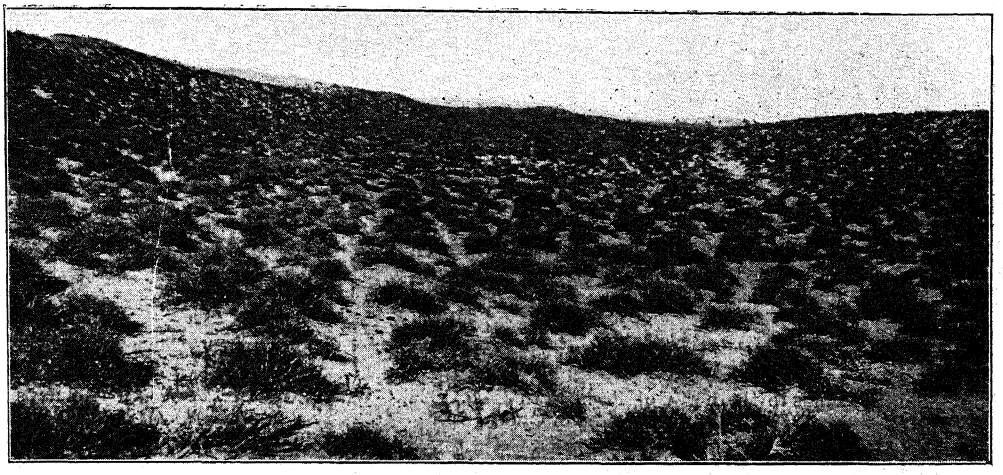


Fig. 1.—Portion of Pasni 'Rek' (Feb. 1933). Vegetation fairly green.

Fig. 2.—Another part of Pasni 'Rek' with high sand hills in the distance (Sept. 1932). Vegetation reviving after rain.

PLATE LXVI.

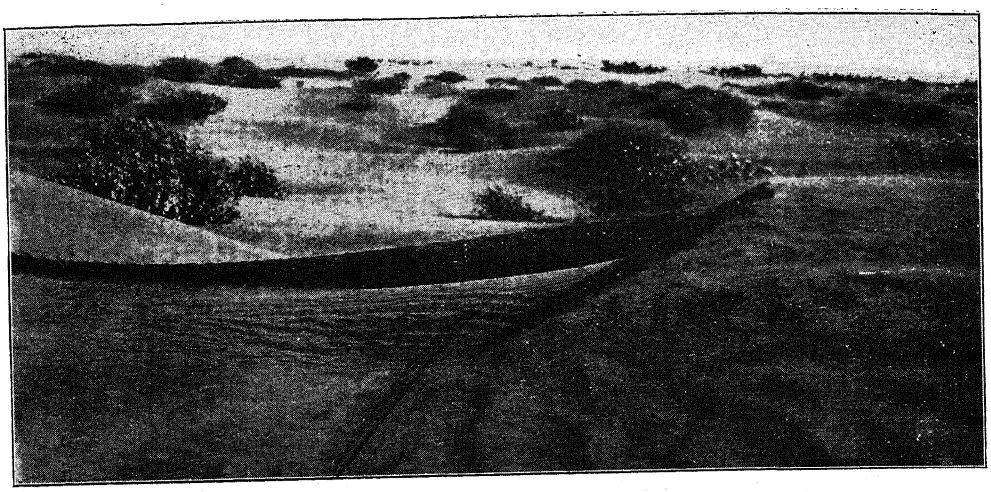


Fig. 1.—Shifting sands to the north of Pasni town (Feb. 1933). With little vegetation except clumps of Calotropis.

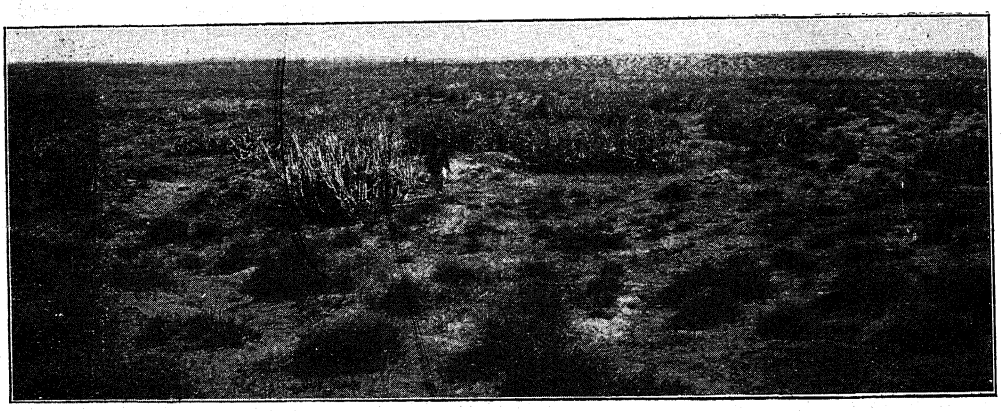


Fig. 2.—'Reks' covering a vast area north of Sonmiani in Lasbela State (Feb. 1932). Tall clumps of *Thuhar* (*Euphorbia sp.*) also found among the desert vegetation.

certain types of sandy areas, thickly covered with scrub vegetation of a xerophytic type, found occurring along the sea-beach on the Mekran coast. These "reks" must originally have been formed, presumably, by the agency of the strong South-West wind, known as *shemal*, that prevails along the coast from April to September, and causes the beach sand to be blown into the interior in the form of slowly moving dunes. The development of the characteristic scrub, with which their surface is now clad, has served to stabilise them, so that they are no longer moving sands now. The vegetation noticeable on these "reks" is composed chiefly of the following plants: 1. 'Marrand'—*Heliotropium ramosissimum* (Boraginaceae)—the plant most preferred by the solitary phase locust; 2. 'Mazoung'—*Frankenia* sp. (?)—(Chenopodiaceae); 3. 'Barshonk'—*Panicum turgidum* Forsk.—(Gramineae); 4. 'Tambo'—*Crotalaria albida*—(Leguminosae); 5. 'Balibur'—*Aerua javanica* Juss.—(Amarantaceae); 6. 'Shalwardir'—*Acanthophyllum squarrosum* Boiss. (Caryophyllaceae); and various annual grasses that spring up after rainfall. All these plants are shrubs not more than a foot or two in height, and, there being no trees anywhere, these "reks" present more the appearance of undulating stretches of meadows than of sandy deserts. The vast areas of these "rek" expanses, which may extend over 30 to 100 square miles along the edge of the sea, have usually been found harbouring locusts, occurring scattered as solitary specimens. At Pasni and Gwadar, they were first noticed by Locust Survey parties in May 1931, and at present, after the lapse of nearly two years, are still to be found in the same areas. On the other hand, at Ormara and Sonmiani—places both similarly situated—locusts were found to have completely vanished for some time in 1932, presumably by reason of dispersion on account of drought, but after the heavy rainfall of July-August, 1932, they have re-appeared. So far as present observations go, there appears to be little doubt that these "reks" function as permanent 'reservations' of the locust.

Climatic conditions of the "reks".

The coastal region of Mekran occurs as a narrow strip of level plain, two to twenty miles broad, stretching from the sea to the foot-hills of the Mekran Coastal Range. Spurs from the latter, however, reach the sea in many places and abut on the sea-shore, so that the coastal plain is broken into discontinuous bits 20 to 50 miles long. The hinterland beyond the Coastal Range is mostly rocky, but contains fairly elevated river valleys, where wheat, *jowar*, and the date palm are cultivated in suitable situations.

The coastal plain offers a contrast to the interior area in its climatic conditions. Along the coast, the winter is much milder owing to the influence of the sea, and the occasional drops of the mercury to 45°F. are due to the in-draught,

in winter, of the chill north wind of Mekran—known as the *gorich*. In summer, the hinterland experiences high temperatures reaching up to 116°F. in the shade, while in the coastal plain the heat is tempered by the strong South-west sea wind—the '*shemal*'—that blows almost constantly, day and night, from April to September, so that the maximum rarely rises above 90°F. Mekran forms part of the Western-Asian winter rainfall area, and most of its light rainfall is received between December and April, during the passage of a series of western disturbances that arise in Iraq and Persia and move on towards North-West India. The heaviest falls occur between December and February, while from March to May, the disturbances are deflected more to the north, so that there are then greater chances of rainfall in the hilly regions of the hinterland than on the coast. During the summer, there is usually very little rain in Mekran proper, whereas the eastern portion of the coast, between Sonmiani and Karachi, is subject to the influence of the Indian monsoon. In certain years, an occasional cyclone may cause the monsoon to extend its sway westward up to Pasni and Gwadar, as happened, for instance, in 1930 and 1932, when heavy falls of about 5 inches of rain were recorded at Pasni.

Observations on locust breeding on "reks".

Given favourable conditions of rainfall, the solitary phase locust would appear to be able to breed both in spring and summer. Actually, breeding took place only thrice during the two years that these areas have been kept under observation. In April-May, 1931, locusts bred after good winter rainfall, and green hoppers were noted; but in July-August, 1931, there were no rains, and consequently no breeding. During the spring of 1932, there was total draught, and no breeding was observed, but in July, 1932, there was very heavy rainfall, and large numbers of green hoppers appeared. As a result of receipt of good winter precipitation in January-February, 1933, small numbers of green hoppers have already been observed.

Actual observations made at the Pasni Field Station during the July-August breeding of 1932 have shown that locusts would begin to breed almost immediately after the receipt of a soaking rain, and that they would not confine themselves to the "rek" areas alone, but would breed in any suitable place even though far removed from the coast. Green hoppers were actually noted during the summer of 1932 at Kandasole, Kappar, and Nokbur, places 5 to 20 miles distant from the coast. Observations made in 1932 also indicate that adults of a new generation could emerge in about two months after egg-laying, and that if favourable conditions of moisture and temperature persist, they would lay eggs without any period of diapause. It appears, therefore, not improbable that if satisfactory rainfall is received early in January during any year, and breeding is set on foot, a second generation would be ready for oviposition by April; and if conditions were

favourable for egg-laying, hoppers of the black gregarious type might come into being in May, and also, by June, fliers sufficiently numerous to begin a migratory flight.

Correlation of rainfall statistics and locust breeding.

The experience so far gained seems to show that there is a very intimate connection between rainfall and breeding in locust economy. Rainfall figures for Pasni and other places on the Mekran coast and Persian Gulf, for the years 1922 to 1926, were therefore collected with a view to find if they would afford some clues as to locust multiplication on the "reks" during these years. Studying the figures (Table II), it is seen that in the winter rainfall period of 1921-22, there was heavy precipitation at Pasni in December 1921 (3·59 in.), but subsequent rainfall was scanty. In 1922-23, on the other hand, there was fairly good rainfall in January, 1923, followed by light showers in February, March and April. Such rainfall would appear to have been favourable for the production of the initial swarms by the end of March. Since there is mention of heavy rainfall on the 6th April in Dasht Niabat, and also a record of 0·95 inch of rain at Turbat in April, it might be presumed that locusts from the Pasni-Gwadar "reks" had migrated into the interior, and given rise to the infestation at Zarrain Bug and Hasadi in Dasht Niabat in April-May, 1923.

Table II.

Winter-rainfall data in inches for stations of the Persian Gulf and the Mekran coast 1922-1926.

Years	Months	Pasni	Turbat	Ormara	Sonmiani	Jask	Bushire	Muscat
1921-22								
	Dec. 1921	3·59	3·14	5·03	1·17	0·86	3·05	0·61
	Jan. 1922	0·89	0·47	..	..	0·23	1·36	0·17
	Feb. ,,	0·26	1·45	0·45	0·49	1·76	0·79	0·25
	March ,,	..	..	..	..	..	..	..
	April ,,	..	..	..	..	..	..	..
1922-23								
	Dec. 1922	0·07	0·68	..	..	1·85	0·77	..
	Jan. 1923	1·41	0·51	3·00	0·25	0·69	5·51	0·26
	Feb. ,,	0·82	1·53	4·50	0·43	0·82	0·40	0·30
	March ,,	0·47	0·27	..	..	0·01	1·40	..
	April ,,	0·10	0·95	..	..	1·68	0·76	1·44
1923-24								
	Dec. 1923	..	..	..	..	..	3·33	..
	Jan. 1924	..	..	..	..	0·25	8·00	0·10
	Feb. ,,	0·70	3·35	1·00	0·35	0·04	1·50	0·21
	March ,,	..	0·49	...	..	..	0·03	..
	April ,,	1·47	1·19	0·14	0·12	..	..	..

TABLE II—*contd.*

Years	Months	Pasni	Turbat	Ormara	Sonmiani	Jask	Bushire	Muscat
1924-25								
	Dec. 1924	0·21	0·44	..	0·04	4·18	13·27	0·73
	Jan. 1925	1·99	1·09	..	..	0·89	2·14	0·27
	Feb. „	1·15	0·07	..	0·04	..	..	0·19
	March „	..	..	..	..	0·13	..	0·19
	April „	..	..	..	..	..	..	..
1925-26								
	Dec. 1925	..	0·07	..	..	..	..	..
	Jan. 1926	7·37	7·83	13·80	0·16	5·14	2·81	0·98
	Feb. „	0·21	0·31	..	..	0·43	1·82	..
	March „	0·48	1·84	..	0·50	0·72	1·32	0·34
	April „	..	..	..	..	0·08	..	0·28

In 1923-24, rainfall was very late and scanty, while in 1924-25, it was comparatively better; it commenced well, but stopped by the end of February. On the other hand, in 1925-26, the precipitation in 1926 was extraordinarily heavy, 7·37 inches having been recorded in the course of 4 days. At the same time, there was similarly heavy rainfall all along the Mekran coast and even in the interior; *e.g.*, Panjgur: 3·69 in.; Turbat: 7·84 in.; Ormara: 13·80 in.; and Jask: 5·14 in. There was some rain at Pasni even in February-March, while in the interior good rainfall occurred in March; *e.g.*, Turbat: 1·84 in.; Panjgur: 1·38 in.; Mand: 2·71 in.; and again in May: *e.g.*, Turbat: 0·49 in.; Panjgur: 0·62 in.; and Mand: 0·81 in. Conditions appear, therefore, to have been favourable for breeding, not only on the coast but also in the hinterland.

In regard to the general inhibitory action of winter temperatures on locust breeding, the following figures extracted from the Monthly Weather Report, for January 1926, of the Indian Meteorological Department, give the actual temperatures that prevailed at Pasni in January, 1926:—

Maximum temperature:	Mean:	74·1°F.	Minimum:	Mean:	52·0°F.
Ditto	Absolute:	80·7°F.	Ditto	Absolute:	47·7°F.

The mean maximum of 74°F. and mean minimum of 52°F. are not so low as to have precluded the possibility of locust breeding. Usually the winter on the Mekran coast is extremely mild, and the low temperatures up to 45°F. sometimes recorded are usually due to the influence of the cold '*gorich*' wind that prevails at times.

It may be noted that during the years 1922 to 1926 there was no summer rainfall of any consequence, and it looks as if there was no summer breeding in these years.

Conclusions.

With the data at present available, it appears to the writer not unreasonable to review the probable sequence of events that had led to the formation of the initial swarms of the great infestation of the year 1926 in Sind as follows. The heavy rains of January 1926 had set the locusts breeding on the Pasni and Gwadar 'reks', and the new brood of adult locusts had probably appeared by the end of March or early in April. The new generation had probably laid eggs again on the 'reks', or migrated to the interior of Kulanch, and given rise to the hoppers of the gregarious phase, and ultimately to the swarms of fliers noted in Kulanch in June-July. These swarms had presumably migrated eastwards, and amalgamating with similar swarms from Ormara side, passed on between June and August towards Kachhi, Lasbela, and Sind, and started the new cycle of infestation that commenced in September 1926 in Sind.

In case this presumption is correct, it would serve to indicate the immense importance of the research work in progress at the new Locust Research Station started at Pasni by the Imperial Council of Agricultural Research, with the object of making a thorough study of the ecology of the solitary phase of the desert locust on the Pasni reks. The experience that may be gained at Pasni in the course of the next two or three seasons may also serve to test the correctness of the premises taken for granted in this thesis.

The Pasni region is important also on account of the fact that it forms part of a much larger area of the same type of locust breeding grounds extending along the coast of the Persian Mekran, and possibly also along the Persian and the Arabian shores of the Persian Gulf, and observations made at Pasni may be expected to indicate, at least to a certain extent, what is happening in the adjoining areas of Persia and Arabia in regard to the development of locust swarms. Such observations may, therefore, possibly serve to give India warning of probable locust incursions sufficiently early to enable her to be prepared to meet locust outbreaks.

Moreover, if future observations lend support to the view that locust outbreaks originate mainly, if not solely, from such 'rek' areas, it may be possible to nip the evil in the bud by poisoning the early broods on such breeding grounds, as was done by Johnston on the Red Sea coast in the spring of 1926. Since, however, such locust 'reserves' occur also in the adjoining Persian territory, and possibly also along the coasts of the Persian Gulf and the Red Sea, the great need of organising locust research, as well as control, on an international basis by the co-operation of neighbouring countries is clearly indicated.

Acknowledgments.

The thanks of the writer are due to Mr. B. P. Uvarov of the Imperial Institute of Entomology, London, for going through an advance copy of this paper and

making certain helpful suggestions, and to the Meteorologist, Karachi, for supplying rainfall data for various stations in Sind and Rajputana, and on the Mekran coast and the Persian Gulf.

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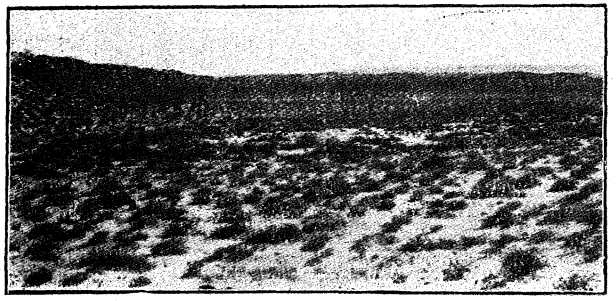

Fig. 1.—Pasni 'rek.'

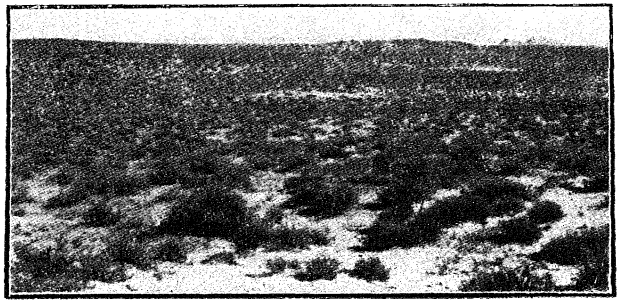

Fig. 2.—Pasni 'rek.'

Fig. 3.—Sonminai 'rek.'

A PRELIMINARY NOTE ON THE BREEDING GROUNDS OF THE DESERT LOCUST (*SCHISTOCERCA GREGARIA* FORSK.) IN BALUCHISTAN.

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(With Plate LXVII).

The history of the recent outbreak of the desert locust in Baluchistan, studied with the help of the available official records from the Baluchistan Niabats, has shown that the locust has been present in Southern Baluchistan since 1923 and that its presence is intimately associated with certain sandy areas known as "reks" situated along the coast line [Rao, 1933]. Under the impression that the coastal area contained some of the breeding grounds of this locust in Baluchistan, the Imperial Council of Agricultural Research opened a Locust Research Field Laboratory at Pasni early in 1932, for the purpose of locating the breeding areas and studying the ecology of the desert locust.

The survey of Southern Baluchistan has revealed the presence of two distinct types of habitats in respect of the desert locust. Along the coast line one comes across long stretches of sandy areas or reks (Plate LXVII) which are quite different from the river beds, valleys and mountains of the hinterland in respect of their geographical, physical, climatic, and biological features.

Physico-climatic features of the hinterland.

The interior of Southern Baluchistan is characterised by the presence of mountain ranges running east to west, somewhat parallel to the coast line and with a mean elevation of about 2,000 feet. They are formed of clay and sandstone cut by hill torrents, are absolutely bare of any vegetation and present a rugged and scorched appearance to the naked eye. Through the valleys in between run small streams and rivers, mostly dried up—the Dasht, the Shadi, the Hingol and the Pohr are the principal ones—all opening into the Arabian Sea. The river beds and the valleys carry coarse gravel and alluvial deposits on their surface and possess at places a thick growth of *Acacia arabica, Tamarix articulata, Capparis aphylla, Zizyphus jujuba, Prosopis spicigera, Nerium odorum and Nanorrhops ritchieana.* The soil of the Kech

and Kolwa valleys is on the whole fertile and a good deal of agriculture is carried on there whenever possible. The annual rainfall is very poor and the general climatic conditions are characterised by very hot and dry summers and cold winters.

Physico-climatic features of the coastal reks.

In contrast to the type of habitat described above, the coastal reks located round about Sonmiani, Kandewari, Dhak, Ormara, Gazdan, Pasni, Gwadar and Pishukan possess physical and bioclimatic features typical for themselves. The reks are discontinuous strips of land situated along the coast line, narrow at one place, wide at another. The surface of these tracts is nowhere plane, but is broken up into ups and downs or undulations, rising to a height of twenty to thirty feet above the sea level. The soil has an upper superficial layer of loose, buff coloured sand, easily blown by the wind and thus exposing at places the underlying hard stratum of sandy rek formed by the mixture of sand and clay, the latter having a very low percentage. The vegetation of these reks is also characteristic. The tree and wood associations of the hinterland are replaced here by bush associations of a xerophytic nature. *Heliotropium ramosissimum. Salsola sp., Lycium barbarum, Crotalaria burhia, Alhagi camelorum, Acanthophyllum squarrosum, Aerua javanica, Atriplex crassifolia,* etc., are some of the common xerophytic bushes found on the coastal reks. Grasses such as *Pennisetum cenchroides, Eragrostis sp., Eleusine aristata, E. flagellifera, Cyperus rotundus,* etc., have also a luxuriant growth in the low-lying areas between the sandy elevations, but only after the rains. The rainfall on these reks, though on the whole poor, is found to be better than in the interior. The annual rainfall records of the last ten years for Pasni give the minimum of 1·38 in. in 1922 and the maximum of 9·25-in. in 1926. During any year the rains are usually received in winter but summer rains though poor in quantity are not infrequent. It should be mentioned here that years might roll on without the hinterland receiving any rains at all, but along the coast line rarely has any year gone by without any winter or early spring or summer rains being received. This fact has a special importance in view of the close relation between the fall of rains and the biology of the desert locust. On account of the nearness of the sea and the blowing of the cool South-West wind, the general meteorological conditions on the coastal reks are much milder than in the interior of Baluchistan.

The sandy nature of the soil which absorbs moisture quickly and retains it to an appreciable extent, the xerophytic nature of the vegetation with its rich water-content, the relative frequency of rainfall at least once a year and the generally moderate conditions of temperature and humidity—these are the principal features of the coastal reks which consequently transformed them into a distinct type of habitat from the point of view of the desert locust.

Locust breeding on the coastal "reks".

As has already been pointed out at the outset of this note, the official records on locust outbreaks show that the desert locust has planted itself in Southern Baluchistan, especially on the coastal reks, more or less permanently. Locust surveys made by the staff of the Locust Research Department of the Imperial Council of Agricultural Research since 1931 pointed out that there has been an intimate connection between the solitary phase of the desert locust and the habitat constituted by the coastal reks. Breeding of the solitary phase of the locust was observed on the reks in the spring of 1931 and since then life-history observations are in progress at Pasni under field conditions. It has also been observed that there is usually a close relation between rainfall and the breeding of the locust. The winter rains of 1930-31 were followed by a fairly good breeding of the solitary phase on the coastal reks. Rains were not received in the winter of 1931-32 with the result that no breeding took place in the spring of 1932. There was heavy rainfall in July-August 1932 and in its wake arose a new generation of the desert locust on all the coastal reks. Early this year (1933) heavy rans were received in the third week of February and in the first week of April. A new spring brood made its first appearance in March and breeding is still in progress in certain parts of the Pasni rek where there is yet (July 1933) enough moisture in the soil. These observations clearly indicate the intimate relationship between the receipt of rains and the breeding of the locust on the coastal reks.

The phases of the desert locust.—The desert locust is believed to be represented by two types of forms or 'phases' distinguishable from one another by morphological and biological characteristics.

The fundamental differences pointed out between the phase refer to the swarming nature of the 'gregarious phase' and the isolated type of life led by the 'solitary phase'. The question of the phases of the desert locust is still in its hypothetical stage and needs to be properly worked out in the light of experiments and observations.

One fact, however, must be noted here, *viz.*, that two types of forms of the desert locust have been observed in Baluchistan, which, even if they could not be distinguished by morphological characters, could easily be separated from one another on the merit of colour variations and general behaviour. The forms met with in the interior of Baluchistan were nearly always of the gregarious type and were evidently the remnants of previous swarms. Those observed on the coastal reks have been invariably of the solitary phase.

Of these phases, the 'solitary' form appears to be the persistent one, while the gregarious phase is only a periodical phenomenon, making its appearance during the period of the formation of the swarms by mass multiplication and the subsequent

migratory flights, and disappearing after the disintegration of the swarming stage. In the light of this distinction, breeding areas of the desert locust exist of which some may be 'permanent', while others only 'temporary'. Limiting the range of locust investigation to Baluchistan, it may be observed that while the interior of Baluchistan can offer temporary breeding areas along its valleys and river beds for the gregarious phase of the locust, during the period of its mass-multiplication and the subsequent invasion, the coastal reks, in consideration of their special physico-climatic features (detailed above), may easily form the most favourable tracts for the persistent presence and breeding of the solitary phase of the desert locust.

Field observations on the life-history of the desert locust made during the summer breeding of 1932 indicate that it takes about six to seven weeks to complete its cycle under favourable conditions. The number of generations produced by the solitary phase usually corresponds with the periods of rainy seasons. Granting that the latter follow each other in quick succession, there is every possibility of an increase in locust population sufficient to cause a mass-multiplication and the subsequent formation of a swarm.

Sandy reks of the type described in this paper appear to be present also in parts of Sind and Rajputana. It is also believed that similar reks may be existing along the coast line on either side of the Persian Gulf.

In conclusion the writer wishes to acknowledge the help and encouragement he received from Mr. M. Afzal Husain, the then Locust Research Entomologist, Lyallpur, and Rao Sahib Y. Ramchandra Rao, the then Deputy Locust Research Entomologist, Quetta, in the course of the investigation of the ecology of the desert locust on the Mekran coast, during 1932, a preliminary account of which is sought to be given in this paper.

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INVESTIGATIONS ON THE DEVELOPMENT OF PRUSSIC ACID IN *CHOLAM* (*SORGHUM VULGARE*).

BY

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(With Plates LXVIII and LXIX and two text-figures).

That *cholam* (*Sorghum vulgare*) under certain conditions of growth becomes highly poisonous for cattle, has been known for a long time past [Benson and Subba Rao, 1906]. In 1902, Peters, Slade and Avery of the Nebraska Agricultural Experiment Station, U. S. A., and Dunstan and Henry in England showed that the poisonous character of the plant was due to the elaboration in it of certain cyanogen compounds capable of decomposing and yielding the poisonous hydrocyanic acid, when masticated and taken into the stomach. The latter authors, in fact [1902], isolated from samples of *Andropogon Sorghum* (called *dhurra*), grown in Egypt, a cyanogenetic glucoside which they named *dhurrin* and which they considered to have the formula $C_{14}H_{17}NO_7$ of para-hydroxy-mandelic-nitrile glucoside. Similar cyanogenetic glucosides were later isolated from other poisonous plants, *e.g.*, lotusin from *Lotus arabicus*, gynacardin from *Gynacardia odorata* R. Br., l-mandelo-nitrile glucoside from *Prunus serotina*, etc. [Robinson, 1930]. A great deal of interest has been shown in recent years in the question of cyanogenesis in plants, especially with reference to grasses and fodder plants capable of causing poisoning among stock, and a surprisingly large number of plants have been found which contain the cyanophoric principle [Robinson, 1930; Finnemore *et al*, 1928; Guerin, 1929, 1930; Seddon, 1930; Rosenthaler, 1929; Couch, 1932, etc.]. Robinson mentions 50 species of plants as producing cyanogenetic glucosides, especially the members of the Rosaceae, Leguminoceae, Graminaceae, Caprifoliaceae, etc. Rosenthaler [1929] gives a list of over 500 plants belonging to the same cyanogenetic category. Various analyses of the prussic acid content in several of these sources have been reported and attempts have even been made to fix roughly the lower value of the prussic acid content at which samples become poisonous for stock. Couch [1932] in a recent publication opines that plants containing 0·02 per cent. and above of potential prussic acid may cause the death of cattle and horses, if as little as 5 lbs. is

eaten. Very few Indian data are available beyond Leather's early work [1906] regarding the prussic acid content of *cholam*, though this is largely used as cattle fodder throughout India, and several deaths of stock have been reported from time to time [Benson and Subba Rao, 1906; Mann, 1919].

But the analytical data reported by the different workers, especially those relative to lethal values, lose much of their significance on account of the widely differing methods of estimation of the cyanogenetic capacity of plant tissues adopted by different workers, which tend to give widely varying results. The difficulty primarily lies in the fact, which has been recognised by several workers [Warth, 1918, 1923; Charlton, 1922, 1926; Robinson, 1929, etc.], that the cyanophoric groups present in poisonous plants may not all be in simple glucosidal forms, but may also be present in several labile combinations, all of which may contribute to the sum total of hydrocyanic acid produced; as such, methods which have not taken this fact into consideration will fail to estimate the total cyanogenetic capacity of plant tissues.

In the present investigation, therefore, which aimed at following the changes occurring in the cyanogenetic capacity of the *cholam* plant, as influenced by the stage of growth and various environmental factors, a considerable amount of attention was first devoted to a satisfactory standardization of the analytical procedure.

Examination of methods.

For estimating the prussic acid content of plant tissues, the Association of Official Agricultural Chemists [1930] recommend the grinding of the material to pass a 20-mesh sieve, macerating with water at room temperature for a period of two hours and then passing a current of steam to remove the liberated prussic acid, which is estimated by one of the following methods:—(*a*) by absorbing it in alkali and titrating against standard silver nitrate or; (*b*) by absorbing it in a known volume of standard silver nitrate and titrating back the excess of silver nitrate with standard sulpho-cyanide; or (*c*) by absorbing the prussic acid in alkali and estimating the cyanide colorimetrically as prussian blue. A preliminary comparison of the methods (*a*), (*b*) and (*c*) with pure cyanide solutions as well as with *cholam* extracts showed that the alkali titration method (*a*) was more suited for the routine analysis of a large number of samples than the other two, and, as it gave results which agreed with those obtained by the other methods, it was generally adopted in most of the experiments detailed below.

But the main analytical difficulty in work on cyanogenesis in plants lies not in the lack of a method for estimating accurately the prussic acid evolved, but rather in the absence of a satisfactory procedure for decomposing and obtaining the cyano-

phoric constituents completely in the form of prussic acid. For this purpose, various devices have been adopted by different workers. The A.O.A.C. [1930] recommend the maceration of the material with water for two hours and subsequent steaming, while Dunstan and Henry [1902] adopted maceration for 12 hours, and Brunnich [1903] for 20 hours. Warth [1918, 1923] and Charlton [1922, 1926] who devoted much attention to this question, tried auto-enzymic action, the addition of brewer's malt, the action of 10 per cent. sulphuric acid, etc.; they found that none of the methods gave the highest value in each case or even concordant results. Recently, Robinson [1929] has recommended a preliminary boiling of the material with water for 20 minutes, followed by the addition of emulsin and removal of prussic acid by aeration.

A comparison of the various methods suggested in the literature showed that none of them gave satisfactory results with fresh *cholam*. The auto-enzyme method, as adopted by previous workers, is defective in as much as varying figures are obtained depending on the time of immersion in water, as shown by the figures in Table I. Higher values were obtained for 48 and 72 hours of immersion, as compared with 24 hours; any period less than 24 hours, like the A.O.A. C's. two hours is obviously insufficient for the complete hydrolysis of the cyanogen compounds.

TABLE I.

Auto-enzyme method.

Sample (100 grms. *cholam*)	Milligrams of prussic acid liberated after			
	Immediate	24 hrs.	48 hrs.	72 hrs.
(1) Field No. 77	2·97	4·86	5·67	11·75
(2) Field No. 67	1·08	4·18	5·94	8·51
(3) Stunted plants	4·19	6·35	8·91	11·17
(4) Normal plants	*nil*	1·09	2·77	2·38

This indefiniteness of time necessary for complete hydrolysis was the chief difficulty met with. It was found that with fresh material, the addition of emulsin or diastase had little effect, and Robinson's method, as applied to *cholam*, is seriously defective in that it recommends a preliminary boiling for 20 minutes, which treatment was found to transform a large portion of the cyanogenetic compounds into a form incapable of yielding prussic acid later, by treatment either with emulsin or with sulphuric acid.

After several experiments, the writer devised the following modification of the auto-enzyme method, which he found to be quite satisfactory in respect of concordance, higher value and certainty of completion of hydrolysis, *i.e.*, definiteness of value. The details of the method are as follows :—

> " A weighed amount of the tissue (about 50-100 grms.) is cut into pieces, pounded well in an iron mortar, water added and the water extract pressed out into a 1,000. c.c. measuring flask through glass wool filter. The operation of pounding and extracting the juice is repeated, adding water each time, till the cold water extract comes to 1,000 c.c. The extracted residue is kept in a beaker and water is added enough to cover it. To the contents of the beaker as well as of the flask is added some toluene or chloroform as preservative. (In this connection it may be noted that contrary to Brunnich's observation, chloroform did not inhibit auto-enzymic action in *cholam*). The auto-enzymic action generally reaches completion in about 24 hours. At the end of this period, 500 c.c. of the water extract and the residue in the beaker, are separately steamed. After 48 hours from the start, the remaining 500 c.c. of the water extract are also steamed. It is usually found that the same value is obtained for the two halves of the water extract. If a higher value be obtained in the second case, it shows the non-completion of the enzymic action in 24 hours ; and so the higher value is taken. When this value is doubled and added to the value of the residue in the beaker, the total prussic acid content of the tissue is obtained."

Table II shows that the enzymic hydrolysis according to the present method is complete in 48 hours, and Table III gives a comparison of the present method with Robinson's method and also the unmodified auto-enzyme method used by Warth, Brunnich and others.

TABLE II.

Water extract method.

Milligrammes of prussic acid obtained from 250 c.c./2,000.

Sample	Immediate	3 hrs.	6 hrs.	24 hrs.	48 hrs.	72 hrs.
(1) Field No. 37	0·9	1·1	1·3	1·7	1·7	1·7
(2) Field No. 77	1·0	1·2	1·7	2·1	2·2	2·2
(3) Field No. 67	1·1	1·4	1·8	2·3	2·3	2·3
(4) Field No. 37	1·3	1·7	2·2	2·5	2·5	2·5

TABLE III.

Comparison of methods.

Sample	Mg. prussic acid corresponding to 100 grms. fresh *cholam*		
	Robinson's methods	Unmodified auto-enzyme method	Author's water-extract method
(1) Second growth *cholam*	5·40	12·15	19·44
(2) Normal plants	*nil*	*nil*	2·70
(3) Emaciated thin plants	1·49	2·97	8·37
(4) Ratoon crop	5·94	16·20	22·95
(5) Secondary shoots	2·84	5·54	10·80
(6) Stunted plants	9·72	18·77	24·03

EXPERIMENTAL.

1. Relation between growth and prussic acid content in cholam.

The method, above outlined, was used in determining the prussic acid content of *cholam* at various stages of growth. The sites of experiments were usually actual fields of *cholam* growing under normal conditions, and the experiments were repeated over two seasons in a number of fields with *Periamanjal cholam* and through one season in different fields with *Chitrai cholam*. The results obtained with *Chitrai cholam* in Field No. 66 are shown in Figs. 1 and 2 and are typical of the results obtained in the cases examined. Expressing the prussic acid content as a percentage of the dry matter, it was found that the percentage was high in the seedling stage (0·2 to 0·3 per cent.) (Fig. 1). A fairly high proportion was maintained till the crop was about forty days old, after which along with the accelerated rate of vegetative growth, the prussic acid content rapidly fell down to 0·02 per cent. and even less. It is interesting to note that the total quantity of prussic acid present per plant shows a continued rise or accumulation in the plant Fig. (2), often amounting to 12 to 15 mg. per plant, till the flowering stage. After the formation of grain, however,

there is a sudden fall in the content of prussic acid, so that the plants become harmless after that stage.

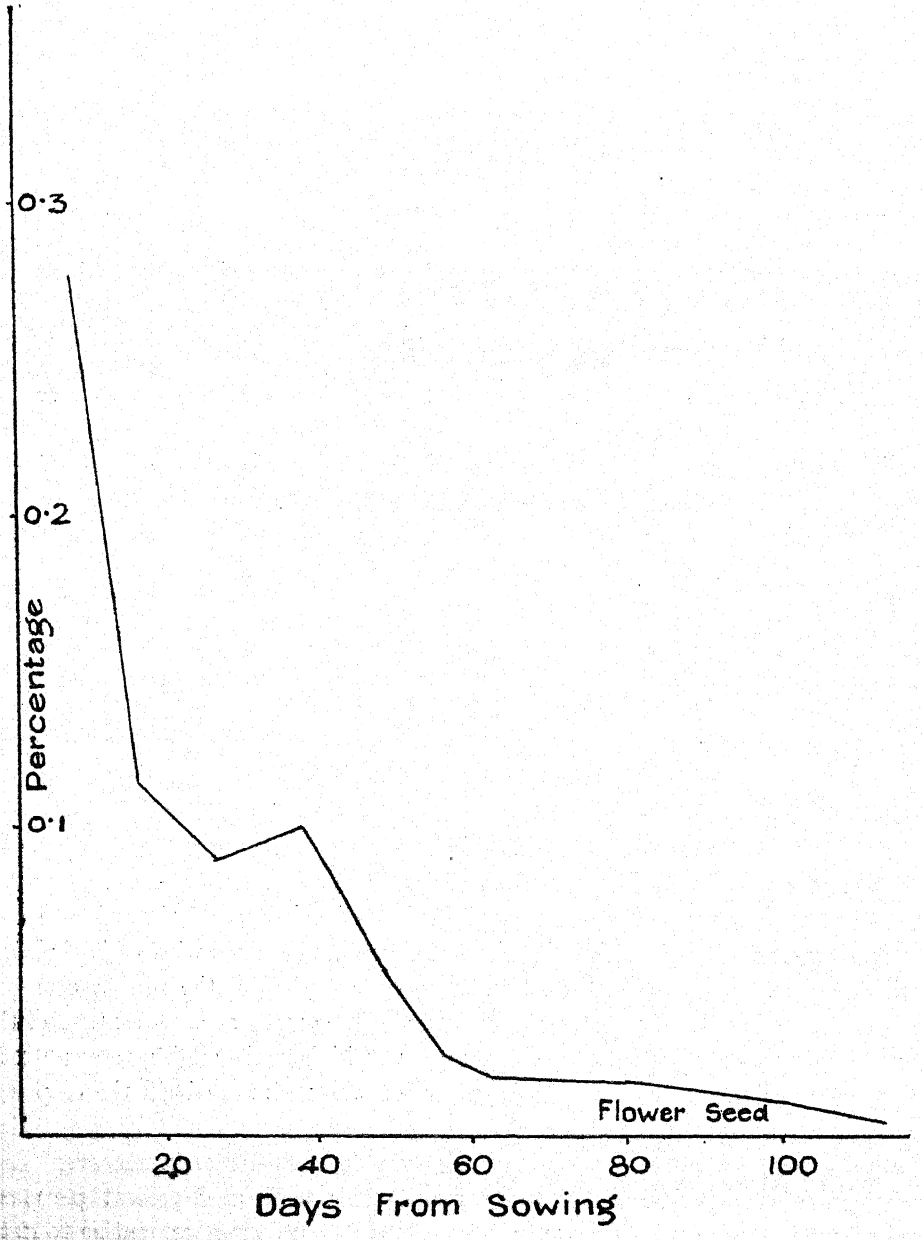

Fig. 1.—Percentage of prussic acid on dry matter.

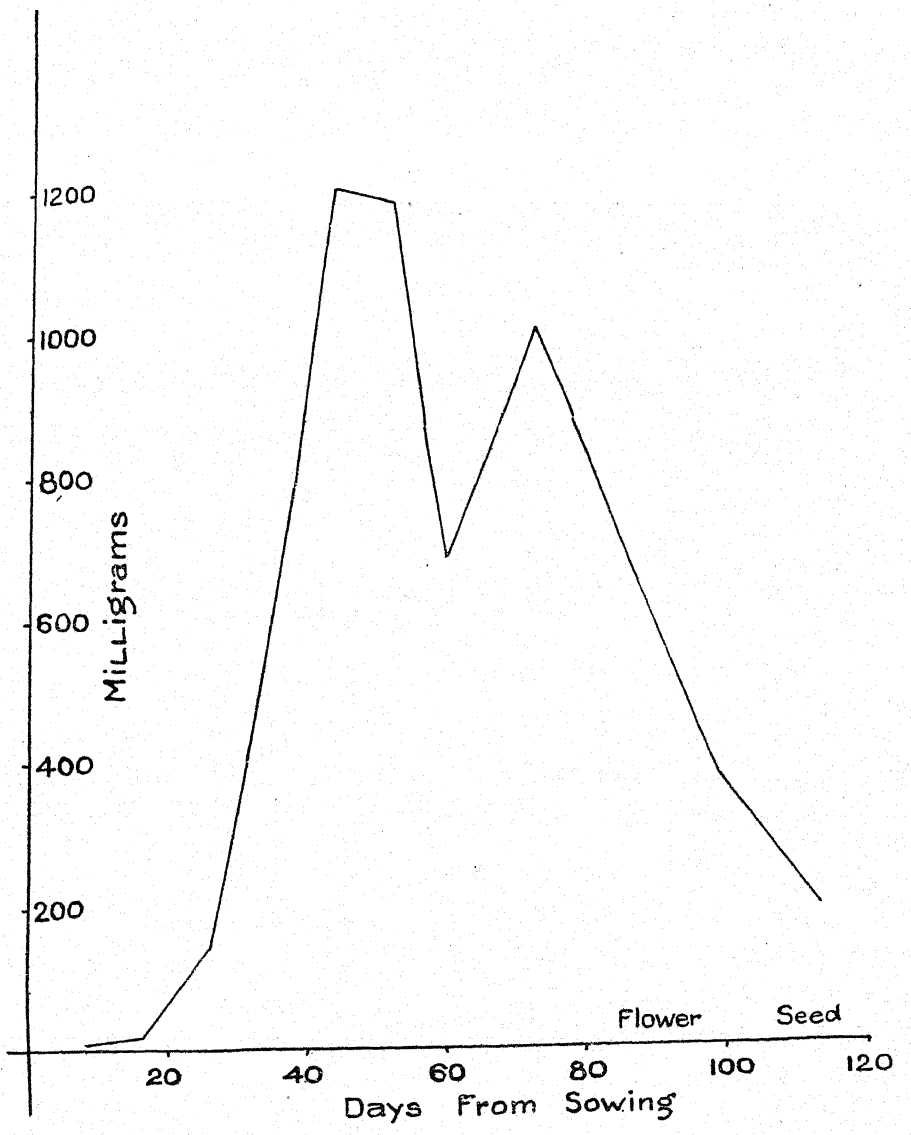

Fig. 2.—Total quantity of prussic acid per hundred plants.

2. Distribution of prussic acid among the tissues.

Some experiments were performed to examine the concentration of the cyanophoric principle in the different tissues of the *cholam* plant, *viz.* Leaves, stem and root. The figures given in Table IV are typical of the results arrived at.

TABLE IV.

Distribution of prussic acid among the leaves, stem and root of cholam.

(Periamanjal cholam, *stunted plants, 1½ months, average weight 50 grams*)

—	Leaves	Stem	Root
	grms.	grms.	grms.
Fresh weight per plant	20	20	6
Dry weight per plant	4·4	2·3	1·3
Mg. prussic acid per 100 grams of fresh matter	13·8	4·9	10·0
Total prussic acid present per plant in the leaves, stem or root	2·77	0·97	0·63

The figures show that the major portion of the cyanophoric compounds (about 60 per cent.) is concentrated in the leaves, which are much more poisonous than the stem. Comparing equal quantities of the leaves, stem and root, on fresh weight basis, the cyanophoric compounds are in the ratio 3 : 1 : 7; and the total cyanogen compounds present in the leaves, stem and root tissues of a plant are in the ratio 9; 3; 2.

3. Changes in prussic acid content at different periods of the day.

An attempt was also made to trace the changes in the prussic acid content of a plant at different periods of the day. Representative samples of plants were pulled out at different hours of the day, and to avoid individual variations, six plants were taken together for a single analysis, and three such repeat samples were taken at each period of examination. The average results obtained are given in Table V.

TABLE V.

Variation of prussic acid content during day time (stunted plants).

Time of day	Mg. prussic acid per 100 grms. (dry wt.)	Mg. prussic acid per plant
	mg.	mg.
6 a. m.	34·7	2·7
8 a. m.	42·3	3·4
10 a. m.	54·2	3·8
12 noon	60·8	4·1
2 p. m.	66·9	4·4
4 p. m.	62·8	4·2
6 p. m.	59·1	4·0
6 a. m.	36·2	2·9

The figures show that the prussic acid content per plant is lowest in the mornings and increases to a maximum at about 2 p.m., after which there is a slow fall till 6 p.m. followed by a rapid decrease in the night. The variation in prussic acid

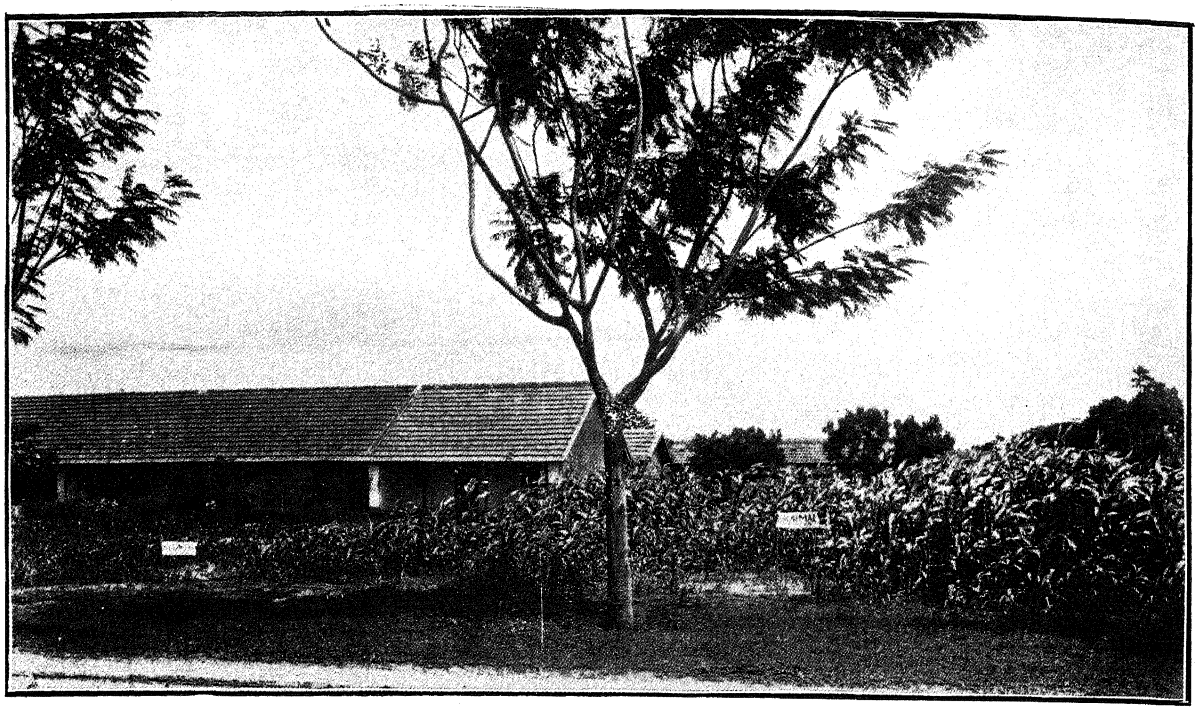

Fig. 1.—Showing (drought) stunted and luxuriously growing plants in Field No. 77, the latter being fed with drainage water.

Fig. 2.—Seedlings, even though grown with plenty water and under optimum conditions, always con-

Fig. 3.—Normal, emaciated and stunted plants. The normal plants were free from prussic acid, the emaciated ones contained a little,

content shows a striking parallelism to the variation in photosynthetic activity and of protein metabolism in the plant, and lends support to the hypothesis that the formation of the cyanogenetic compounds is a normal part of the protein metabolism of the plant.

4. Effect of environmental conditions on the prussic acid content of cholam.

A field of *Periamanjal cholam* suffering from serious drought and highly stunted (Field No. 77), was taken up for examination, and its prussic acid content compared with that of *cholam* growing in an adjacent part of the same field, which had been supplied with drain water and had grown up luxuriantly (Plate LXVIII). The stunted plants showed nearly 6 to 7 times the percentage of prussic acid present in the normal plants (Table VI). This effect of drought was examined in a number of other fields also, and in each case it was noticed that drought is one of the factors promoting the accumulation of prussic acid in *cholam*.

TABLE VI.

Comparison of normal and stunted plants.

Sample (100 grms. dry matter)	Mg. prussic acid liberated by normal plant	Mg. prussic acid liberated by stunted plants
	Mg.	Mg.
(1) Field No. 67	0·68	11·07
(2) Do.	1·35	10·39
(3) Do.	0·67	13·77
(4) Field No. 77	1·35	8·36
(5) Do.	2·83	11·17
(6) Do.	0·81	10·40
(7) Do.	0·27	6·35

Another factor is intensity of sunlight. Plants which had grown up rapidly in the shade had a much lower content of the poison than plants growing in direct sunlight. In both the cases (drought and sunlight), slow growth was associated with a higher amount of prussic acid, while rapid growth was associated with less. But that light is not a necessary condition for the elaboration of prussic acid in *cholam* was shown by growing *cholam* in pots in darkness, when the etiolated seedlings, a week old, showed about the same percentage of prussic acid as the control seedlings grown in light (Table VII). Also, insufficient water (drought) may increase the amount of prussic acid, but cannot account for the whole of it present as shown by

the fact that seedlings even though grown with plenty of water and under optimum conditions, always contained prussic acid (Plate LXIX).

TABLE VII.

	Percentage prussic acid on dry matter		Percentage prussic acid on dry matter
Periamanjal cholam plants growing in sunlight, 40 days old, contained . . .	0·046	*Chitrai cholam* seedlings 1 week old, grown in light .	0·289
Periamanjal cholam plants, growing in the shade, 40 days old, contained . . .	0·025	*Chitrai cholam* seedlings 1 week, old grown in darkness.	0·338

It would therefore seem that the elaboration of the cyanogen compounds in *cholam*, as one of the products of the nitrogen metabolism of the plant, is an inborn characteristic of the plant, which may be modified in quantity but cannot be suppressed by controlling the external conditions. That cyanogenesis in *cholam* is, in fact, a genetic character, seems probable from an interesting experiment carried out by Moodie and Ramsay [1929], who crossed Sudan Grass, a non-poisonous plant, as the female parent, against *Sacchaline Sorghum* as the male parent, and found that the offspring, which had all the appearance of the Sudan Grass, contained prussic acid and was highly poisonous to cattle. Recent literature shows that an increasing number of species of plants [Finnemore, 1928, 1932; Robinson, 1930; Guerin, 1929, 1930; Rosenthaler, 1929; Heilbronn, 1929; Floriani 1929; Ramsay and Henry, 1929; Hagen, 1930; Mirande, 1932; Seddon, 1930; Couch, 1932, etc.] have been found to be capable of elaborating cyanogen compounds, which indicates that such elaboration of cyanogen compounds may not be a special characteristic of particular groups of plants, but might be a common feature of all nitrogen metabolism in plants, the cyanogen compounds playing the necessary intermediary role in the synthesis of proteins, according to the theory advanced by Treub [1896; *cf.* also Robinson, 1929, Menaul, 1921, and Stekelenburg, 1931].

5. Ratoon crops.

It is the general experience of farmers that ratoons and secondary shoots are much more poisonous than the first crop. This is to be expected since the ratoons generally grow during a period of hot weather and represent new growth of a stunted nature. By an actual examination of the ratoons growing in a dozen fields

Fig. 1.—Secondary shoots (tillering) in *cholam*, due to abundant food supply.

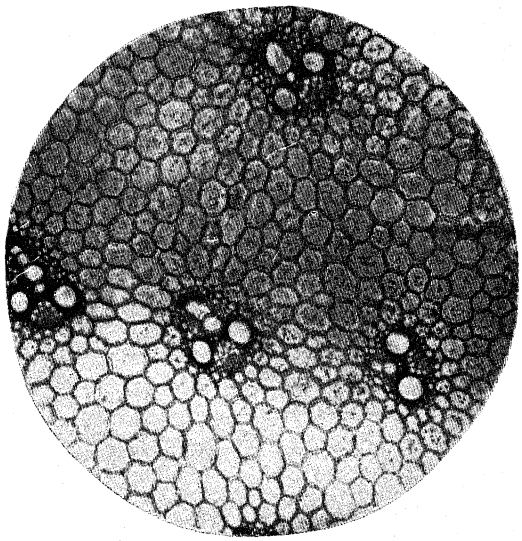

Fig. 2.—Transverse section of the stem of a young ratoon. (Note the abundance of starch.)

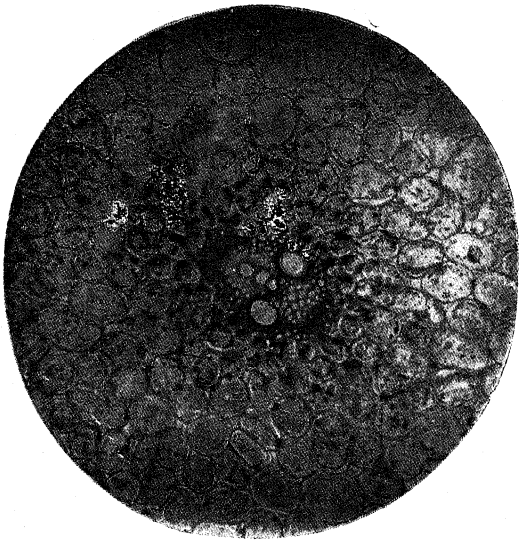

Fig. 3.—Transverse section of the stem of a stunted plant. (Note the abundance of starch grains.)

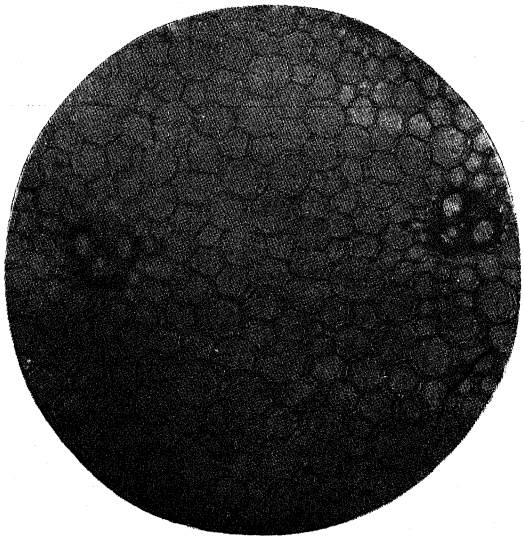

Fig. 4.—Transverse section of the stem of a normal plant. (Note the abundance of starch.)

at different stages of growth and in different seasons, it was found that the ratoons contained a much higher percentage of prussic acid than normal first growth of the same height and age (ratoons 15-20 cm. high showed a prussic acid content of 0·15 to 0·2 per cent. on dry matter). The shorter the ratoon, the greater is the percentage of the poison; as the ratoon grows longer, the percentage falls down, but at each stage, the amount of prussic acid present in a ratoon is much higher than that in a normal plant. The concentration of poison in a particular field of ratoon is no doubt dependent on several factors like humidity, temperature, fertility of the soil, the vigour of the first crop, etc. The more vigorous the first crop and the more sugar and starch it had accumulated in the stubble, the greater is the elaboration of the poison in the second growth.

An interesting case that came under experiment was a crop of *Periamanjal cholam* grown in Field No. 77, which, owing to the supply of drain water, had received such an abundance of food that after the main earhead had ripened, several fresh shoots began to grow from the various nodes, and some of these also began to grow ear. These side shoots can be termed "secondary shoots" in contradistinction to "second growth" from the stubble, after the crop is cut. An example of such tillered growth (secondary shoots) is shown in Plate LXIX. It was found on analysis that the main stem was free from starch and almost free from prussic acid, while the secondary shoots were rich in both (0·099 per cent. as against 0·002 per cent. in the main stem).

6. *Effect of after-treatment on the amount of prussic acid in* cholam.

Where *cholam* is grown for grain, it is usual to grow it close at the beginning and later, after about a month, to thin out. At present, such thinnings are generally discarded for fodder on account of their high content of prussic acid, and are generally thrown into the pit or heap for the preparation of manure. But the thinnings, about 30 or 40 days old, are very rich in mineral nutrients and proteins, and form a valuable concentrate of high feeding value, especially in our country where most fodder is poor in the necessary mineral constituents. As such, an attempt was made to see whether by any simple means in the nature of an after treatment, it was possible to decrease the prussic acid content of these thinnings to within safe limits which could warrant their use as fodder. Some of the treatments tried were :—(*a*) drying in the shade; (*b*) drying in the sun; (*c*) drying in the oven at 100° C.; (*d*) treatment with dilute sulphuric acid for a few minutes, and (*e*) silaging. The data obtained (Table VIII) show —

(*a*) Drying in the shade had little effect on the amount of prussic acid generated. The enzyme which brings about the hydrolysis of the cyanogen compounds with

the production of free prussic acid was still active and produced amounts of prussic acid which were only slightly below those obtained from the fresh plants.

TABLE VIII.

Effect of after-treatment.

Quadruplicate samples		Percentage prussic acid on dry matter			
		I	II	III	IV
(1)	No treatment	0·096	0·102	0·082	0·114
	After drying in shade	0·082	0·091	0·064	0·092
(2)	No treatment	0·115	0·098	0·076	0·106
	After drying in sun	0·078	0·032	0·046	0·061
(3)	No treatment	0·078	0·104	0·112	0·101
	After heating at 100° C.	0·008	0·010	0·006	0·011
(4)	No treatment	0·056	0·095	0·124	0·116
	After application of 10 per cent. sulphuric acid	*nil*	*nil*	*nil*	*nil*
(5)	No treatment	0·104	0·112	0·096	0·106
	After silaging	0·004	0·005	0·002	0·007

(*b*) Drying in the sun was distinctly better. Even now, the enzyme was not completely destroyed, as shown by the fact that tissues kept in water for intervals of 4, 12, 24 and 48 hours, showed progressively increasing liberation of prussic acid. But the total amount liberated was much less, in several cases less than half the amount liberated in the fresh stuff. Still a good amount was left behind and the question whether what is left behind is still poisonous for cattle or not, can only be settled by actual feeding trials. In this connection it may be noted that Leather [1906] found that on sun drying the fodder did not decrease the amount of cyanogenetic glucoside in it. He observes "On this point, there is no doubt. Brunnich made a series of comparative experiments to test it, and in the Laboratory of the Government of India, we have found similarly that the glucoside is not changed by this process."

(*c*) In contra-distinction to treatments (*a*) and (*b*), it was found that heating the tissues for several hours at 100°C., almost completely removed the cyanogenetic compounds. Only inappreciable amounts of prussic acid were liberated, even though the fresh tissues were highly poisonous. But, obviously, this treatment is not economically feasible on the large scale and is of merely theoretical interest as showing the destructive action of heat at this temperature on the poison and the enzyme.

(*d*) Treatment with dilute sulphuric acid (10 per cent.) was found to be very successful in destroying the whole of the prussic acid, and in leaving behind a residue free from poison. It was noteworthy that during the treatment with acid and on subsequent distillation with steam, no prussic acid was liberated, showing that the 10 per cent. sulphuric acid acting on the cyanogenetic compounds converts them into a form incapable of liberating prussic acid. The non-liberation of prussic acid was not due to the hydrolysing action of sulphuric acid on prussic acid after its liberation from the tissues, as shown by the fact that quantities of prussic acid from 0·5 mg. to 10·0 mg. added to the tissues in the form of prussic acid, were recovered quantitatively in presence of 10 per cent. sulphuric acid. Even 5 per cent. sulphuric acid was found to behave as 10 per cent. sulphuric acid, in its destroying action on the cyanophoric principle. This destructive action of sulphuric acid (dilute) is noteworthy and is apparently contradictory to the current ideas, based upon Dunstan and Henry's original experiments [1902], that the cyanogen compounds in *cholam* are present in the form of the glucoside " dhurrin " which is hydrolysed by acids or emulsion, yielding prussic acid. The action of acids, in the present investigation, in destroying the cyanophoric principle, appears to be almost instantaneous and might probably be due to the effect of a change in pH, whereby the poisonous principle is rendered inocuous. It is suggested that pH might serve a similar controlling influence in the living plant in converting the intermediary cyanophoric bodies into other harmless products. As regards the application of the acid method on the large scale, it is worth examining whether the sprinkling of some suitable acid, *e.g.* acetic or hydrochloric over a mass of poisonous *cholam* and keeping for some time, with frequent stirring of the mass, will destroy the poisonous element.

(*e*) But the most promising solution of the cyanogen question from the fodder point of view, seems to lie in the method of " silaging " it. The effect of silaging, on the prussic acid content of *cholam* was examined in two seasons in 1930 and 1931, with samples of about 1,500 lbs. of *Periamanjal* and *Chitrai cholam* varieties, which were silaged in pits in the ground 3 ft.×3 ft.×3 ft. The samples taken for silaging were what are usually considered to be dangerous stuff, *viz.*, second growth crop (ratoon) and thinnings, containing well over 0·1 per cent. of prussic acid on dry matter. It was found that silaging for 2 to 3 months, removed nearly the whole of the poisonous element, decreasing the prussic acid content to about 0·005 per cent. This decrease is attributed to the effect of increased acidity in silaging, similar to the action of 5 or 10 per cent. sulphuric acid, but a part of the effect may also be due to enzymic action.

The silages in all cases were fed in bulk to cattle at the rate of 30 to 40 lbs. daily, were relished by them and caused no disorder or trouble. Thus, silaging the

cholam thinnings or second growth or stunted plants for a period of two to three months, can safely be recommended as an effective method for the destruction of the poisonous element in these plants, and for their utilization as a concentrated feeding stuff, low in fibre and rich in nitrogen and mineral nutrients.

7. *Classification of the cyanogen compounds in* cholam.

It has been noted above that when poisonous *cholam*, rich in cyanogen compounds, is subjected to differing treatments, the amount of prussic acid liberated varies greatly, showing that the cyanophoric groups may be present in a mixture of different types of combinations. During the course of experiments carried out to test this point, it was noted that :—(1) Immediate steaming, without allowing time for auto-enzymic action, liberated only a fraction of the total prussic acid, leaving a residue from which no further prussic acid could be obtained by acid or enzyme treatment, while (2) auto-enzymic hydrolysis in presence of water, gave a much higher yield of prussic acid, and (3) addition of dilute sulphuric acid to fresh *cholam* tissue, destroyed the cyanophoric group completely and no prussic acid was evolved ; (4) but, if auto-enzymic action be allowed to take place for some hours and then dilute sulphuric acid is added and steam-distilled, an amount of prussic acid is obtained corresponding to the degree of auto-enzymic hydrolysis that had taken place.

In this connection may be mentioned the work of Warth [1918, 1923] and Charlton [1922, 1926] on Burma Beans, who after a laborious volume of work found that the cyanogen compounds appeared to exist in several different forms and combinations, among which they distinguished between (*a*) auto-enzyme prussic acid, (*b*) glucosidal prussic acid, and (*c*) total prussic acid, which contained other combinations in addition to the above two forms.

From a large number of experiments performed on widely differing samples of *cholam*, like thinnings, stunted plants, second growth, secondary shoots, etc., and a comparison of the yield of prussic acid obtained by different treatments, it was found that the cyanogen compounds present in *cholam* tissues could be classed under the following two heads :—

(*a*) *Labile cyanogen compounds*, which are decomposed by steaming at 100°C., with or without adding water, with or without cutting the tissue to pieces. The decomposition can also be brought about by the enzymes present in the tissue. It is found that a very large proportion (60 to 75 per cent.) of the cyanogenetic compounds present in young seedlings, belongs to this class and is liberated by steaming. That such production of prussic acid on steaming was not due to the effect of cutting the tissue to pieces and the consequent liberation of the enzyme and its rapid decomposing action on the glucoside, before steaming could destroy

the enzyme, was shown by the fact that when steam was passed through the whole uncut seedlings (15-20 days old), the same amount of prussic acid was liberated as when the tissues were previously cut. That the effect was not due to the degree of moisture content, was shown by the fact that when more water was added to the tissues before steaming, the same or slightly smaller amount of prussic acid was liberated, leaving behind the same residual fraction unacted upon by this treatment (25 to 30 per cent.).

This liberation of free prussic acid from fresh *cholam* seedlings by simple steaming, contradicts Dunstan and Henry's failure to obtain such free prussic acid by steaming of Sorghum (Dhurra) tissues. On the basis of their failure to obtain such liberation of free prussic acid, they controvert Greshoff and Treub's view (quoted by them) that in many tropical plants, prussic acid occurs as such in the free state. But numerous repetitions of the writer's experiments have always shown that when fresh, young *cholam* is steamed, an amount of prussic acid is liberated, whose quantity depends on the poisonous nature of the plant. The writer, however, cannot agree with Greshoff and Treub that prussic acid is present in the "free state", for the reason that when 10 per cent. sulphuric acid is added to such poisonous plant tissues containing a large amount of the so-called "free prussic acid", not even a trace of prussic acid is obtained on steaming. It has been already shown that 10 per cent. sulphuric acid has no appreciable hydrolysing action on "free prussic acid" and that quantities of 0·5 to 10·0 mg. of prussic acid added to the tissues in the form of potassium cyanide before the addition of sulphuric acid, yielded quantitative recoveries of prussic acid added. "Labile prussic acid" therefore is not "free prussic acid", but is probably some unstable cyanogen compound, which easily gives up prussic acid on steaming, but is converted into non-cyanogenetic forms on the addition of acid. The proportion of labile prussic acid to total prussic acid is greatest in the seedling stage, becomes lower with growth, reaches a minimum (almost zero) at the time of flowering and then tends to rise again.

(*b*) The proportion of prussic acid other than labile prussic acid, we can conveniently denominate as "bound prussic acid". This is hydrolysed by the enzymes present in the plant itself, yielding prussic acid. The action of heat (100°C.) on the "bound prussic acid" is different from that on the labile prussic acid. Heat (100°C.) destroys the "bound prussic acid" once for all, without any liberation of prussic acid. It undergoes some transformation, whereby it is rendered incapable of generating prussic acid subsequently either by enzymic (emulsin) or acid hydrolysis. The effect of acid, however, is similar in the two cases. Ten per cent. sulphuric acid destroys the "bound prussic acid" as well as the "labile prussic acid". In air-dried tissue, where diastase is not very vigorous, the addition of

diastase to the tissue increases the total amount of prussic acid liberated, and this increase seems to come mainly from the "bound prussic acid" fraction.

After a piece of poisonous *cholam* tissue has been kept in water for an hour or two, there are, therefore, at least three forms of combination in which prussic acid is present:—

(1) "Free prussic acid", formed by enzymic hydrolysis of "labile prussic acid" and "bound prussic acid", after the tissue is cut and kept in water for some time. It is not destroyed (but is recovered quantitatively) on adding 10 per cent. sulphuric acid and steaming.

(2) "Labile prussic acid", present in poisonous *cholam* to a large extent. Decomposed and liberated from tissues by simple steaming. Destroyed by 10 per cent. sulphuric acid.

(3) "Bound prussic acid", calculated by subtracting (1) and (2) from "total prussic acid" content of the plant tissue, as found by the writer's "water extract" method. Not evolved on heating, but destroyed; liberated by auto-enzymic action; in dry tissues, the liberation is hastened by adding diastase; destroyed by adding 10 per cent. sulphuric acid.

8. *Simple test for poisonous* cholam.

The writer has, throughout the present work, found a close relationship between the accumulation of starch and that of prussic acid in poisonous *cholam*, for which no satisfactory explanation has yet been found. All plants rich in prussic acid like young *cholam* seedlings, plants stunted due to drought, second growth *cholam* (ratoon) and secondary shoots (referred to on p. 861), are also rich in starch in their stem, while the normal plant, after the seedling stage, is almost free from starch in the stem. This difference can therefore be utilized to distinguish between poisonous and non-poisonous plants in a simple way.

The test is carried out by cutting a transverse section of the stem, near the bottom node, staining with iodine and examining under the microscope. Plants highly poisonous, are stained blue or black to the naked eye, and a microscope is unnecessary. Such plants generally contain above 0·1 per cent. prussic acid on the dry matter. The writer has found this relationship between starch and prussic acid to apply even at different growth periods of the same plant. In the early seedling stage, there is starch present in the stem, it decreases rapidly with vegetative growth and is almost absent at the flowering stage. After seed-maturity, however, starch again makes its appearance in the stem. It has been already noted (page 855) how a similar variation also takes place in the amount of prussic acid present in the plant. This is a curious relationship and can be explained either by supposing that the cyanogen compounds are combined with the starch, loosely in the "labile prussic

acid" and more firmly in the "bound prussic acid", or that the presence and accumulation of starch and prussic acid in the plant are both guided by the same controlling influence in the biochemical processes of the plant's life. The matter awaits further elucidation, but as it is, it can be used by the farmer as a simple and valuable test for finding out whether a particular field is poisonous to his cattle or not. He pulls up a few representative plants, takes transverse sections near the bottom node (thick sections will do), and applies a little iodine solution and observes the slide against a white background with the naked eye. If the solution is coloured black or blue, the plants are dangerous and contain over 0·1 per cent. of prussic acid on the dry matter. If there be no apparent staining, the slide may be observed under the microscope. If there be a good many starch grains visible, stained blue or black, gathered especially round the xylem vessels, the plants contain prussic acid, but not to a dangerous extent (below 0·1 per cent.); whereas, if the section is fairly clear, the cattle can be fed on the field without any fear. After a few hours' practice, any farmer can learn to do the above testing for himself.

Summary.

(1) A satisfactory method is described for the estimation of the cyanophoric content of poisonous *cholam* (*Sorghum vulgare*).

(2) The prussic acid content of a normal crop of *cholam* decreases from the early stages (0·2 to 0·3 per cent.) progressively, till the flowering stage, at which it can be considered harmless.

(3) The leaves contain about 60 per cent. of the total cyanogen compounds present in the plant, and contain a higher percentage on the dry matter than the stem or root.

(4) The total prussic acid content of a plant and the percentage on the dry matter, are lowest in the morning, then increase up to about 2 p.m., after which they show a slight decline till 6 p.m., followed by a rapid decline in the night.

(5) Young *cholam* seedlings less than 40 days old, plants stunted due to drought, second growth or ratoons, and secondary shoots, are found to have the highest content of prussic acid (0·1 to 0·2 per cent.) on dry matter. Seedlings grown in darkness showed as high a percentage of prussic acid as those grown in sunlight.

(6) Drying in the shade had little effect in decreasing prussic acid content (decrease only about 10 per cent.). Drying in the sun, showed only partial decrease (30 to 40 per cent.). Heating the tissues to 100° C., and maintaining at that temperature for some hours destroyed the poisonous principle. Immersing poisonous *cholam* for a short time in 10 per cent. sulpuric acid was found to effectively destroy the cyanophoric group.

(7) One of the best methods for rendering poisonous *cholam* innocuous for feeding purposes, is to silage it. Silages prepared from *cholam* thinnings (seedlings), and second growth (ratoons), originally rich in prussic acid (0·1 to 0·2 per cent.), gave only a trace of the poison, after two months silaging; it is suggested that such silaged thinnings and second growth may form a valuable feeding stuff, low in fibre and rich in protein and mineral nutrients.

(8) There is evidence to show that the cyanophoric group present in *cholam* may not be wholly in the glucosidal form, but may also be present in less stable combinations, and an attempt has been made to classify the different groups.

(9) A simple iodine test has been given for differentiating between poisonous and non-poisonous *cholam*, based on the accumulation of starch in the stem of poisonous plants.

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EFFECT OF MOSAIC ON THE TONNAGE AND THE JUICE OF SUGARCANE IN PUSA, PART III.

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In continuation of the last two seasons' experiments [McRae; 1931, 1932] thirty-six plots each 5×56 yards were laid down in adjacent pairs in the order mosaic-free, mosaic-infected, mosaic-infected, mosaic-free and so on. Planting was done on the 17th February 1932 and the area was good, light land suitable for growing sugarcane. For the mosaic-infected plots sets were cut from canes whose leaves had the mosaic markings at the time of planting while for the mosaic-free plots sets were cut from canes that from frequent observations during the growing season were known to be entirely free from mosaic disease.

During the season 1932-33 a small amount of infection spread to the mosaic-free plots and seven clumps altogether were found to have the disease in six plots. Thus the mosaic-free plots remained substantially free from mosaic disease throughout the season. This small amount of spread during the season agreed with the result got in another plot where 34 varieties of mosaic-free cane were grown in alternate rows 33 yards long with mosaic-infected Co. 213 canes and only 10 varieties became infected. *Saretha* had seventeen, *Chunee* three, Co. 210 fifteen, Co. 213 eight, Co. 304 twenty, Co. 312 one, Co. 313 forty-five, Co. 327 one, Co. 332 one and Co. 337 two clumps that showed the characteristic mottling on the leaves.

On the 29th March, 14th April and 5th May 1932 the number of shoots was counted and the percentages calculated on the number of buds on the sets planted taking three buds to a set. The percentage germination after six, eight and eleven weeks is as follows :—

TABLE I.

Percentage germination.

Plot No.		1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	33	36	Mean
		2	3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	34	35	
29th March 1932	F	24	24	28	32	29	30	29	27	29	24	28	25	32	33	30	32	29	26	28·4
	M	26	27	31	30	31	29	31	27	26	27	32	30	37	31	31	30	26	26	29·3
14th April 1932	F	28	25	32	33	33	31	34	33	32	30	36	32	34	37	38	35	34	30	32·6
	M	29	33	34	34	34	32	32	33	30	29	35	37	38	42	38	35	27	31	33·5
5th May 1932	F	49	66	77	88	80	71	86	82	88	71	86	83	90	100	100	89	86	63	81
	M	61	69	63	78	71	74	78	76	71	73	73	80	100	100	100	95	75	70	79

F represents mosaic-free and M mosaic-infected cane.

Between the last two counts the plot had to be irrigated with water in which a little crude oil emulsion was mixed in order to reduce the attack of termites so that it is likely that the total germination was higher in May than it would normally have been, had the field been unirrigated. On the whole the germination was very good and the difference in the means for the two series had no statistical significance.

One of the limiting factors in carrying out this experiment is the presence of insects that destroy the cane. The following notes give a record of the main facts and show that on the whole the attack of insects was evenly distributed over the plots.

In the hot weather from the middle of April to the end of June the cane was attacked by the shoot borer *Scirpophaga nivella*. The bored shoots were removed and the percentages are shown in Table II.

TABLE II.

Percentage of borer attack in May and June.

F	M	F			M			F	M	F			M		
Plots		May	June	Total	May	June	Total	Plots		May	June	Total	May	June	Total
1	2	9	14	23	7	10	17	20	19	8	3	11	7	2	9
4	3	8	8	16	9	10	19	21	22	4	3	7	5	3	8
5	6	8	9	17	7	5	12	24	23	5	4	9	3	3	6
8	7	7	5	12	7	4	11	25	26	5	3	8	4	3	7
9	10	6	5	11	9	8	17	28	27	3	3	6	4	2	6
12	11	8	5	13	7	6	13	29	30	4	6	10	4	4	8
13	14	6	4	10	6	4	10	32	31	5	2	7	4	4	8
16	15	7	3	10	6	4	10	33	34	5	4	9	4	4	8
17	18	8	2	10	9	2	11	36	35	4	4	8	5	5	10

The mosaic-free plots had an average of 10·9 per cent. of shoots bored while the others had 10·5 per cent. The difference is not statistically significant.

During the five months from April to August egg-masses of *Scirpophaga nivella* and Pyrilla species were collected regularly and the numbers are given in Table III.

TABLE III.

Egg-masses collected from May to August.

		Scirpophaga nivella										Pyrilla species									
Count		1st	2nd	3rd	4th	Total	1st	2nd	3rd	4th	Total	3rd	4th	5th	6th	Total	3rd	4th	5th	6th	Total
Plots F	M	F					M					F					M				
1	2	5	..	2	..	7	9	..	2	1	12	..	4	5	3	12	..	3	4	4	11
4	3	7	..	3	1	11	14	..	2	2	18	..	3	4	3	10	..	..	5	5	10
5	6	21	..	3	..	24	15	..	2	..	17	..	6	3	3	12	..	5	5	4	14
8	7	21	..	3	..	24	14	..	2	..	16	..	6	4	4	14	..	5	4	3	12
9	10	16	..	4	..	20	22	..	1	..	23	..	5	6	8	19	..	3	7	9	19
12	11	16	..	1	..	17	13	..	2	1	16	..	3	6	6	15	..	1	5	5	11
13	14	12	1	3	..	16	29	2	2	2	35	..	2	4	4	10	..	2	8	6	16
16	15	16	2	1	..	19	12	2	3	1	18	..	3	6	7	16	..	1	4	5	10
17	18	25	2	1	..	28	20	1	..	1	22	..	2	5	4	11	..	2	3	5	10
20	19	17	1	1	..	19	24	2	..	..	26	..	3	5	8	16	..	2	4	4	10
21	22	19	2	1	..	22	12	3	2	..	17	..	1	2	10	13	..	2	4	9	15
24	23	13	4	1	..	18	12	3	2	..	17	..	2	4	12	18	..	1	5	11	17
25	26	13	3	2	..	18	16	2	2	..	20	..	2	3	10	15	..	2	3	11	16
28	27	4	2	3	..	9	6	2	2	..	10	..	2	2	6	10	..	1	4	9	14
29	30	9	3	2	..	14	10	2	..	1	13	..	3	6	10	19	1	4	4	7	16
32	31	6	3	2	..	11	8	2	..	..	10	..	3	4	10	17	3	4	5	6	18
33	34	8	4	2	..	14	6	1	2	..	9	..	3	3	5	11	..	4	10	4	18
36	35	6	1	..	..	7	8	2	..	..	10	3	2	7	8	20	3	2	9	5	19
Mean						16·5					17·1					14·3					14·2

The top shoot borer apparently ceased to lay eggs on the advent of the rains in early July. After the 22nd of August no further attempt was made to collect egg-masses of Pyrilla because the canes were rather tall. Three species of Pyrilla have been recorded on sugarcane at Pusa, *P. aberrans* Kby., *P. pusana* Dist., *P. perpusilla* Wlk., but no attempt was made to distinguish to which of them the various egg-masses belonged.

In neither case is the difference in the means between the two series of plots statistically significant.

During the months of August and September termites attacked the canes in greater numbers than usual at this time of year owing to the scanty rainfall. The attacked plants were removed and a dilute mixture of crude oil emulsion was

applied to the soil at the places from which the plants were taken. The following table gives the number of clumps removed from each plot.

TABLE IV.

Number of plants attacked by termites in August and September.

F	1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	33	36	Mean
M	2	3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	34	35	
F	106	66	99	71	49	104	62	80	103	41	49	45	58	77	62	80	97	62	73
M	85	70	104	71	63	141	81	70	80	73	61	50	63	75	87	52	110	68	78

The difference of the means of the mosaic-free and mosaic plots is not statistically significant.

No damage was done by cane diseases caused by fungi as there were no stem diseases and the leaves were remarkably free from spots and there was none caused by animals, the field being surrounded by a fence, proof against jackals, pigs and porcupines. At harvest time the plots were examined under the direction of the Imperial Entomologist, and Table V gives the percentage of insect infection. Only the first, third and fifth rows in each plot were examined. The percentage was calculated on the number of canes.

TABLE V.

Percentage of insect infection at harvest.

F	M	F				M			
Plots		Top shoot borer	Stem borer	Root borer	Termites	Top shoot borer	Stem borer	Root borer	Termites
1	2	25·5	6·6	9·6	21·6	32·03	5·3	12·09	14·4
4	3	42·8	7·1	14·7	22·2	30·4	7·06	10·5	11·5
5	6	32·3	5·2	9·7	9·6	34·4	4·9	10·2	10·7
8	7	34·07	6·2	10·09	11·6	34·2	4·1	10·8	7·03
9	10	33·4	4·8	12·8	8·5	34·4	4·7	10·1	8·8
12	11	29·3	4·6	9·7	10·1	41·0	4·7	10·9	9·8
13	14	29·1	3·5	8·5	5·2	31·6	3·5	9·3	3·7
16	15	32·1	3·02	9·2	4·5	24·01	3·6	8·7	4·4
17	18	27·3	3·9	10·9	5·1	29·7	3·9	10·3	5·5
20	19	23·2	4·02	6·7	3·8	24·3	3·3	7·5	5·08
21	22	22·4	3·4	8·3	4·2	20·9	2·9	8·8	8·0
24	23	18·1	3·2	9·1	7·7	23·5	4·8	8·9	9·5
25	26	19·0	2·7	8·2	7·6	21·3	4·06	8·1	8·2
28	27	17·4	4·3	7·9	6·8	19·5	4·6	8·5	8·6
29	30	22·7	4·9	7·0	9·2	20·8	6·7	7·8	8·4
32	31	22·1	3·5	6·6	12·5	20·08	3·08	7·2	9·2
33	34	21·02	4·2	8·05	10·1	18·4	3·8	7·7	11·9
36	35	16·6	3·3	10·3	11·7	20·6	3·5	8·3	9·8
Mean		26·02	4·35	9·29	9·5	26·7	4·36	9·2	8·58

The top shoot borer is *Scirpophaga nivella* Fabr., the stem borers are *Argyria sticticraspis* Hmpsn., *Diatræa venosata* Swinh., and *Chilo zonellus* Swinh., and the root borer is *Emmalocera depressella* Swinh.

The mean difference in each case is small and has no statistical significance, so that whatever loss was caused by these insects was so evenly distributed that it did not have any effect on the difference of the mean tonnage of the plots. The bored shoots were not weighed separately as such a task was beyond the powers of the staff while harvesting.

At harvest gaps of three feet or more in the rows of cane were measured and the following table gives in feet the sum of the lengths of all the gaps in each plot.

TABLE VI.

Gaps in the rows at the time of harvest.

4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	33	mean
3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	34	
124	87	85	89	100	125	131	110	170	111	110	113	105	100	126	111	112
105	106	104	121	127	101	102	137	116	141	157	142	91	135	75	104	116

The difference between the means of the two series is not statistically significant. The total length of the gaps is nearly 15 per cent. of the total length of the rows. If it be assumed that, had there been living canes in the gaps, the weight of such cane in each plot would have been proportional to the weight of cane in the rest of the plot, then the weight of the actual cane in each plot represents 85 per cent. of the potential weight. The difference between the actual and the potential weights would be eight maunds per plot or at the rate of 150 maunds per acre. This may be taken roughly as a measure of the loss due to the gaps which are caused chiefly by termites, much less by root borer and to a very small extent by other causes.

Samples of cane were taken for analysis just before harvest from the 20th to the 25th January when the cane was considered to be ripe. The sample from each plot consisted of 5 yards of cane taken at random from each of the five rows and the cane was crushed in the small, bullock-driven, three-roller iron mill. The analysis of juice was furnished by the Chemical Section and the details are as follows :—

TABLE VII.

Samples taken from 20th to 25th January 1933.

Plot Nos.		Weight of cane in lbs.		Weight of juice in lbs.		Percentage weight of juice to cane		Brix corrected		Sucrose per cent.		Glucose per cent.		Purity per cent.	
F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
4	3	301	322	201	213	66·7	66·3	16·59	17·77	12·68	14·24	1·04	0·85	76·44	80·15
5	6	261	344	173	210	66·3	61·1	17·73	17·69	14·60	14·39	0·86	0·78	82·32	81·32
8	7	419	431	315	298	75·2	63·4	17·59	17·37	14·20	13·87	0·86	0·86	80·71	79·82
9	10	380	377	279	272	73·5	71·9	17·76	17·75	14·34	14·45	0·85	0·78	80·73	81·39
12	11	386	336	282	223	72·9	66·4	18·37	17·90	14·90	14·41	0·84	0·81	81·10	80·52
13	14	408	430	278	297	68·6	69·1	16·93	17·43	13·18	14·08	1·06	0·83	77·83	80·74
16	15	483	456	333	276	68·9	60·5	17·71	17·74	14·22	14·33	0·95	0·93	80·29	80·75
17	18	407	386	279	254	68·7	65·9	17·46	17·86	13·94	14·60	0·99	0·84	79·85	81·74
20	19	346	427	237	294	68·4	68·8	17·69	17·34	14·20	13·73	0·95	0·92	80·28	79·18
21	22	420	391	292	279	69·5	71·1	17·62	18·30	14·08	14·97	1·04	0·85	79·92	81·81
24	23	367	353	252	237	68·6	67·3	18·07	18·33	14·58	14·80	1·06	0·91	80·67	80·75
25	26	397	374	274	258	69·0	68·9	17·81	17·03	14·22	13·33	1·04	1·06	79·86	78·37
28	27	422	379	293	261	69·4	68·9	17·01	16·36	13·31	12·32	1·16	1·16	78·24	75·29
29	30	439	311	311	212	70·8	68·1	17·36	18·50	13·27	15·01	1·17	0·86	76·46	81·12
32	31	364	405	248	283	68·1	69·7	18·53	18·39	15·23	14·94	0·74	0·79	82·16	81·24
33	34	370	377	256	252	69·2	66·9	18·91	17·93	15·64	14·40	0·67	0·93	82·71	80·30
Mean		385·6	381	268·9	257·4	69·61	67·14	17·696	17·73	14·16	14·24	0·95	0·88	79·97	80·28
		—4·6		—11·5		—2·47		+·034		+·08		—·07		+·31	

At the time of harvest between 26th January and 3rd February, 1933, three yards of cane from the ends of the rows and five rows of cane from the two extreme sides were removed to eliminate edge effect. Thus there were left in each plot five rows of cane in an area of 5 yards by 50 yards. Before harvesting the cane it was noticed that the stand of cane at the extreme ends of the block was much thinner than in the rest of the block. Termites in the latter part of the growing season had been more active there. A row of low trees had been cut out in the previous season along the roads a few yards from the sides of the block, and experience in Pusa has shown that termites are liable to be more numerous till the roots decay and it was considered that the two end plots, both mosaic-free, did not show the relative difference in yield between their corresponding mosaic plots that might be due to the presence of the disease. It was considered that the unequal damage caused by white ants, that had destroyed the cane, was likely to mask the difference due to disease. It was accordingly decided that leaving the two end pairs of plots out of account would provide a set of 16 pairs that would be a truer representation of the facts. Thus 15 rows of cane were cut off from the two ends of the block.

The weight of canes in all the plots was recorded in pounds avoirdupois on a new machine but each has been calculated into maunds to have the record comparable with those of the two previous reports on this subject. The weights in maunds of stripped cane from the 32 plots are as follows, one maund being equal to 82·28 lbs. avoirdupois.

TABLE VIII.

Weight in maunds of stripped cane.

F	M	F	M
4	3	38·26	38·58
5	6	41·70	44·20
8	7	47·72	45·69
9	10	45·53	40·53
12	11	42·39	40·31
13	14	47·98	49·90
16	15	46·16	49·43
17	18	47·63	46.98
20	19	47·26	52·41
21	22	52·92	47·84
24	23	52·45	49·90
25	26	50·53	48·99
28	27	50·30	51·42
29	30	47·73	45·55
32	31	42·85	45·16
33	34	41·00	41·86
Mean		46·40	46·17
Mean difference		·23	

The calculated weight of stripped cane per acre, 890 maunds, is considerably above the average yield of fields on the farm but still many single acres on the farm gave a higher yield than this. Compared with the cane-growing area of the farm, the land on which the experiment was conducted is considered to be only fairly good and as the cultural and manurial treatment was the same, it is likely that the good yield is due to the partial protection from the depredation of insects given in removing bored shoots and egg-masses and in the attempt to keep down termites as well as the protection from the usual damage caused by jackals, pigs and porcupines. These operations were carried out by coolie boys under the supervision of an active fieldman and, except perhaps against termites, both as regards personnel and cost they are within the compass of any cane grower. Otherwise the conditions are typical of much of the cane-growing land in North Bihar.

The weight of cane in each row was taken and the differences calculated leaving only one row, then two rows and then three rows in each plot for possible edge effect but the results were the same as for the five rows. There was no statistical significance in the differences between the means. By Fisher's test also there was no statistical significance in the differences in the mean yields. The coefficient of variability was low, being 8·7 in the mosaic-free plots and 9·8 in the mosaic plots.

The figures to determine the statistical significance of the difference in yield between the two series of sixteen pairs of plots are summarised below :—

TABLE IX.

Statistical constants.

Co. 213	Mean difference	Standard deviation	Mean difference / Standard deviation	Odds
Cane	·23	2·8	·08	0
Juice	11·5	38	·3	6 : 1
Percentage of juice to cane	2·47	3·64	·67	77 : 1
Calculated juice per plot	1·3	2·6	·5	27 : 1
Brix	·034	·51	·06	0
Sucrose	·08	·84	·1	2 : 1
Glucose	·07	·12	·58	36 : 1
Purity	·31	2·1	·15	2 : 1

Thus the difference in the percentage of juice to cane, the calculated juice to cane, and the glucose are alone statistically significant. This year then in the mosaic plots there was no loss in the weight of stripped cane and the quality of the juice had not deteriorated, indeed there was slightly less glucose. However, 4 per cent. less juice was extracted from the cane in the mosaic plots.

Intensity of infection in North Bihar.—In July 1932 an attempt was made to get a better appreciation of the intensity of mosaic infection in the cane of North

Bihar. The Deputy Director of Agriculture, North Bihar Range, co-operated in the survey by allowing three overseers of his staff to work with three fieldmen of the Mycological Section at Pusa and by planning the details of the tour. Their method of sampling was to take narrow strips through various fields in each of the sixty-five localities and to record the total number of clumps of cane and the number of clumps infected, in order to get the percentage of infection.

As some growers who allowed their cane to be examined do not wish their estates to be named, only the number of localities in the districts is given. On looking at a map on which the places examined are plotted we believe that the sample as a whole presents a fairly representative picture of the position in North Bihar with regard to the intensity of mosaic disease. A summary of the record is as follows:—

TABLE X.

Intensity of mosaic disease on cane in North Bihar.

Variety	Districts	Localities	Area of strips examined	Infected clumps	Percentage infection
Co. 213	Chapra	5	4·33	60	0·1
	Gopalganj	12	6·6	758	0·9
	Siwan	7	4·4	2,775	4·8
	North Muzaffarpur and Champaran	13	18·95	162	0·06
	North-East Muzaffarpur and Darbhanga	10	16·09	60	0·03
	Total	47	50·37	3,815	0·58
Co. 210	Chapra	..	..	..	..
	Gopalganj	5	11·8	281	0·7
	Siwan	13	8·14	37	0·03
	North Mazaffarpur and Champaran	3	3·5	2	0·05
	North-East Muzaffarpur and Darbhanga	6	2·93	2	0·04
	Total	27	26·37	322	0·09
Co. 285	Chapra	1	1	0	0
	Gopalganj	1	·25	87	2·6
	Siwan	1	3	2	·006
	North Muzaffarpur and Champaran	2	1·25	..	0
	Total	5	5·5	89	·12
Co. 205	Muzaffarpur	1	·13	11	·7

The increase in the percentage of mosaic in Co. 213 in 1932 is due to the high infection in three localities in the Siwan Sub-Division consisting of 1·76 acres with 12 per cent. infection. The Coimbatore canes have almost completely ousted the local variety in the area examined but in two localities of ·64 acre the latter was found to be wholly infected with mosaic disease.

The summary of the record for this and the two previous seasons in which a survey was made is as follows :—

TABLE XI.

Infection of cane in North Bihar.

Variety	1927			1931			1933		
	Area of strips examined	Number of localities	Percentage infection	Area of strips examined	Number of localities	Percentage infection	Area of strips examined	Number of localities	Percentage infection
Co. 213 . .	39·5	6	0·03	13·6	8	0·2	50·37	47	0·58
Co. 210 . .	5	1	0·5	1·6	5	0·96	26·37	27	0·09
Co. 205 . .	6·7	4	15·0	1	4	9·0	0·13	1	0·7
Co. 285 . .	Not grown on a field scale.						5·5	5	0·12

With the exception of Co. 205 the percentage of infection in sugarcane of the Coimbatore varieties in North Bihar is still low. That in Co. 205 has decreased but this may be more apparent than real as the sample was very small and as Co. 205, because of its high fibre content, is discouraged by the mills, the area under this cane is decreasing rapidly and its place is being taken by Co. 285. It appears that the disease in Co. 213 has slightly increased in the five years 1927 to 1933. That it can increase considerably is shewn by the high percentage of infection in Siwan. It is probable that the infection on Co. 210 has slightly decreased. Mosaic disease has not yet been found on Co. 214.

On reviewing the results of the last three seasons' experiments one sees that no factor that has been measured has varied consistently in all three tests except the calculated juice from cane.

Co. 213	1931	1932	1933
	Per cent.	Per cent.	Per cent.
Cane	—4·6	—14·8	*
Percentage juice to cane . . .	*	*	—2·47
Calculated juice	—4·8	—15·4	—4
Brix	—·81	—·58	*
Sucrose	—·99	—·67	*
Glucose	*	*	—·08
Purity	—1·56	*	*

It is true that, whenever in any season the difference of the means of a measured factor has had statistical significance, it has been in the direction of disadvantage for the mosaic cane except in the less amount of glucose in 1933. This is the position so far and the experiment will be carried on for at least two more years.

Summary.

On comparing mosaic-free and mosaic-infected sugarcane in sixteen pairs of plots, each 5 by 50 yards, after sufficient cane to eliminate edge effect had been removed, and on analysing the measures for weight of stripped cane, weight of juice, brix, sucrose, glucose and purity, it appeared that in the season 1932-33 about four per cent. less juice was extracted from the mosaic-infected cane and in it the glucose was slightly less and that none of the differences in the other measures was statistically significant.

The difference in the germination of the means of the two sets of plots was not statistically significant.

The cane was attacked uniformly by various insects and at the time of harvest about forty per cent. of the canes showed some form of insect attack.

During the last three seasons no factor that has been measured has varied consistently in all three with the exception of the calculated juice from cane and this has always been less in the mosaic-infected plots.

Whenever the difference in the means of measured factors has had statistical significance, it has been in the direction of disadvantage to the mosaic-infected cane except in the less amount of glucose in 1933.

This investigation was carried out partially by a grant from the Imperial Council of Agricultural Research for the investigation of mosaic and other sugarcane diseases.

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AN ALTERNARIA BLIGHT OF THE LINSEED PLANT.

BY

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(With Plates LXX and LXXI and one text figure).

I. INTRODUCTION.

During the months of January and February of 1928, the writer had observed a severe form of blight of the linseed crop (*Linum usitatissimum* L.) in some of the experimental plots in the Botanical Research Farm at Cawnpore. An undetermined species of *Alternaria* was found to be associated with this blight [Dey, 1929]. In the following years reports of its occurrence, received from the various localities, indicated its prevalence throughout the United Provinces. This year the disease appeared particularly severely in the Government Experimental Farm at Gorakhpur and in the Cawnpore Research Farm, in badly drained plots where water remained stagnated for a few days after heavy showers of rain. In the latter place it was more or less absent from the high, well-drained fields. Generally speaking, on average fields the loss caused by this disease to the crop as a whole is not great but in low-lying ill-drained fields the damage may be considerable. Tables I and II, which were compiled from the figures supplied by the Economic Botanist (oil seeds) Cawnpore and the Deputy Director of Agriculture, North Eastern Circle, Gorakhpur, respectively, show the extent of damage to the crop caused by the blight in the affected fields. The linseed rust (*Melampsora lini* D. C.) was almost absent at both the places this year, and in these fields no other disease was noticed. The loss in yield might thus be ascribed entirely to *Alternaria*.

TABLE I.

Loss in yield of linseed caused by the blight in badly diseased plots at Cawnpore Research Farm, 1933.

Variety No.	Yield per acre from healthy plots—calculated from average of 5 plots of 1/80 acre each	Yield per acre from diseased plots—calculated from average of 5 plots of 1/80 acre each	Percentage of loss
1150	17 mds. 5 srs.	12 mds.	29·9

TABLE II.

Loss in yield of linseed caused by the blight in badly diseased plots at Gorakhpur Experimental Farm, 1933.

Variety No.	Yield per acre from healthy fields—calculated from average of 4 plots of 1/40 acre each	Yield per acre from diseased fields—calculated from average of 2 plots of 1/40 acre each	Percentage of loss
1206	5 mds. 32 srs. 8 ch.	4 mds. 7 srs. 8 ch.	27·9
1162	4 mds. 32 srs. 8 ch.	2 mds. 35 srs.	59·6

II. SYMPTOMS.

The disease was easily noticeable at the time of flowering, after a spell of wet weather. All parts of the plant above ground were affected, particularly the buds, flowers and the upper leaves. The first indication was the failure of the flowers to open during the day. Minute dark brown spots appeared near the base of the calyx. They enlarged, deepened in colour and spread all over passing into the pedicel. The petals and other floral parts shrank and rotted away. The unopened flowers ultimately shrivelled and dried up, projecting through the neck of which could be seen the remains of disorganised petals. The flower buds similarly turned black near the pedicel, the discolouration spreading over the calyx above and into the stem below. These also collapsed and dried up. Similarly in the young erect leaves, the dark brown spots starting near the base spread over and passed into the stem. They dried up and got twisted or curled outwards. In the older leaves, the tips of which hang downwards, the attack usually started at the tip. The affected leaves broke off easily from the stem. The concentric rings in the lesions, so very typical of the attack of *Alternaria* on other hosts, were altogether absent.

After a shower of rain, the water remained entangled in droplets for sometime within the bases of the calyx and erect leaves, which offered suitable conditions for the germination of spores of the causal organism. Hence the infection started at such points. The flowers were more to suffer, as in closing at night they enclosed the droplets of water within them, which thus remained there for several hours, and thereby offered a far more favourable condition for the attack. The places of infection of the disease in the plant, as well as its severity in the undrained fields where the air round the plants remained wet for a long time, showed the intimate relation of the disease with humid condition.

In severe attacks the whole plant dried up, the stem turning greenish black nearly upto the base, and bearing on it the blackened distorted dry leaves and the dark brown shrivelled-up buds and flowers (Plate LXX, fig. 1). If the attack

Photo by P. K. D.

Fig. 1.—Blighted shoots of linseed plants.

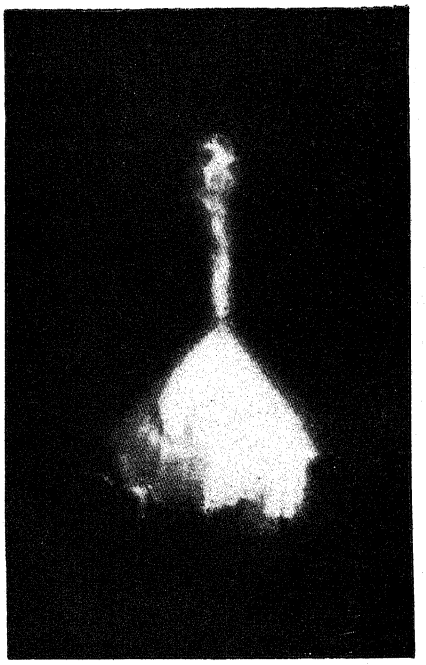
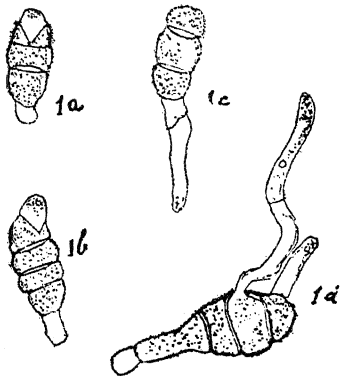
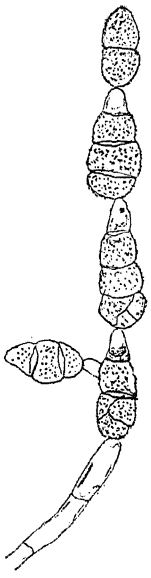


Photo by A. K. M.

Fig. 2.—Gynæcium of a linseed flower, taken 48 hours after the stigma was inoculated with the spores of *A. lini*. The cottony growth of the mycelium containing chains of spores can be clearly seen in the upper half of the style through which the infection spread.

PLATE LXXI.





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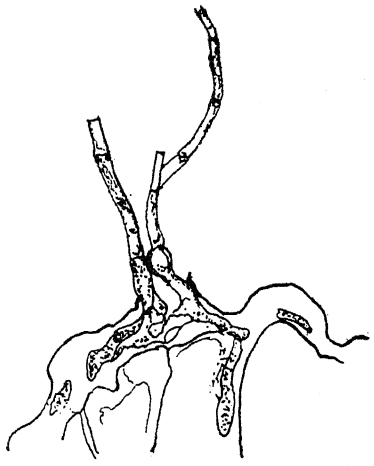

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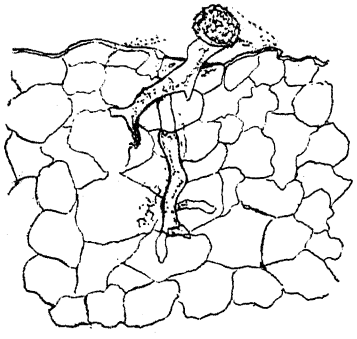

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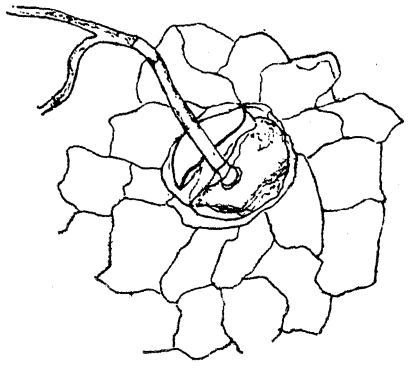

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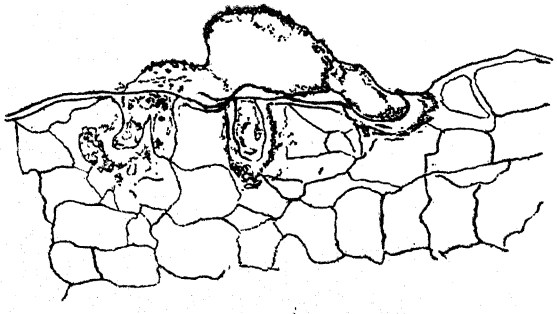

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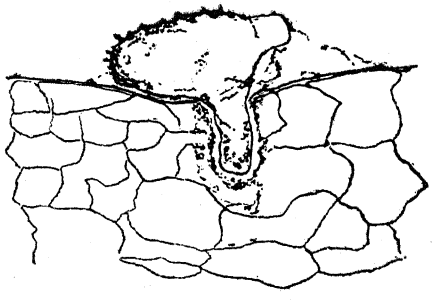

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Dey — Alternaria lini

PLATE LXXI.

Fig. 1 a-c.—Germinating spores of *A. lini*. In Fig. 1c the germ-tube has emerged from the extremity of the beak-cell. ×700.

Fig. 1d.—A germinating spore of *A. lini* stained with dilute gentian violet. The crack in the episporium is distinct. The endosporium has elongated with the germ-tube to form its outer wall. ×700.

Fig. 2.—A chain of spores of *A. lini*. The beak-cells have thinner and smooth wall. One of the spores is bearing a secondary one. ×700.

Fig. 3.—Two sporophores have emerged through a crack in the cuticle of the leaf. The epidermal layer is much swollen, due to clustering of hyphæ within it. The sporophores have swollen bases. ×500.

Fig. 4.—A sporophore has come out through a stomata. Its swollen base is clear. ×500.

Fig. 5.—The hypha has penetrated deep into the tissue of the petal, and has branched. The material was fixed eight hours after inoculation. ×500.

Fig. 6.—The germ-tube has just penetrated into the petal through a rupture in the cuticle, the outward margin of which is bent upwards and the inward margin downwards, due to pressure of the entering hypha. There is a clearer area immediately round the tip of the germ-tube indicating enzymic action. Two other germ-tubes, probably from the same spore, have also penetrated. Their connection with the spore cannot be seen in the section. ×1,000.

Fig. 7.—Same as *Fig. 6*.—The spore became firmly fixed to the surface of the petal by means of 'mucilage', the remains of which are seen as granular threads and beads. The germ-tube has entered vertically downwards. The hole in the cuticle is wider than the diameter of the tip of the germ-tube. Dissolution of the host tissue is seen as a halo round the tip. ×1,000.

started a little later in the season, when the pods had already formed, the latter were attacked like the flowers at the base of the sepals and spread into the pod and the stem. The pods turned prematurely brown and dried up.

If the moist weather continued for some days, the affected flowers became covered with a white downy mycelial growth. This, however, changed very soon into sooty-black colour due to the production of spores, long chains of which could be seen even with the naked eye. Ordinarily the affected parts of the plant soon became covered with a velvety black bloom, which could be rubbed off with fingers or shaken off like dust.

The microscopic examination revealed pale olive, aerial mycelium of the fungus, bearing chains of septate spores of *Alternaria*, which covered the surface of the affected parts. These aerial sporophores arose from the almost colourless vegetative mycelial threads which permeated through the completely disorganised host tissue and clustered in knots in the epidermis below the cuticle. The epidermal layer was thus considerably swollen. The sporophores usually burst through the cuticle (Plate LXXI, fig. 3), two or more emerging through the same opening, or sometimes came out singly through the stomata (Plate LXXI, fig. 4). At the point of origin from the mother hypha, the sporophores were slightly swollen into a small vesicle, agreeing in this respect with *A. macrosporium* [Jones, 1928]. Plate LXXI, fig. 3 is a drawing from 8 μ thick microtome section stained with iron-alum hœmatoxylin and Plate LXXI, fig. 4 is that from a hand section, which was fixed and stained by Amann's fluid containing 0·5 per cent. cotton-blue [Linder, 1929].

That this fungus *Alternaria* was the cause of the blight was confirmed by subsequent studies.

III. Causal fungus.

The spores of the fungus from the surface of the blighted plants were picked up with the sterilized needle and sown on 5 per cent. rice agar medium in Petri dishes. They were incubated at 85°F. Since the spores were lying loosely in chains on the surface, it was easy to obtain pure cultures by this method. On the second day white colonies, about 5 mm. in diameter, appeared. Fresh cultures were prepared by transferring the hyphae from the extreme edges of these colonies into fresh media. On the fourth day the colonies were 5-6 cm. in diameter, showing distinct zonation of dark greenish brown rings alternating with pale olive areas, except near the edges where the colour remained white. The dark colour was due to the appearance of spores. After 5-7 days, the whole of the colony, which by now had completely filled the 9 cm. diameter Petri dishes assumed a sooty black colour, especially in the central regions, with the whole surface looking dry and velvety.

(*a*) *Mycelium.*

The mycelium consisted of distinctly separate, vegetative and reproductive parts. The former was almost colourless with a faint greenish tint, appearing grey-white in mass, sparsely septate—the septa being more numerous in the older hyphae—and filled with coarsely granular protoplasm. Here and there could be seen small swellings or vesicles, which exhibited at times a circular streaming movement of their granules. This vegetative mycelium remained submerged in the medium, the hyphal branches running more or less parallel to one another towards the margin of the colony, without forming a dense weft. The hyphae varied in thickness between 2μ and 7·5μ with the average diameter of 4μ.

After about two and a half days, the older vegetative hyphae began to throw aerial filiform branches or sporophores, which grew straggling several millimeters long, frequently sending out, in irregular succession, simple racemose lateral branches. The exact length of the sporophores could not be measured owing to the fact, that by the time they stopped lengthening and started bearing spores numerous other sporophores and their branches appeared in the field, completely obliterating the course of a single sporophore. The sporophores were olive-brown in colour, with a deeper shade near the extremities. They were more closely septate, especially at the ends, which were sharply cut up into small cells. The main axis, as also each of the lateral branches, stopped growing after a while, and their tips began to change into spores. With the stoppage of growth in length, still other side branches grew out from the main axis.

The sporophores thus consisted of long filamentous aerial, branched hyphae measuring 2·5μ to 4·5μ in thickness with the average of 3·5μ.

(*b*) *Spores.*

The spores were in chains, dark olive in colour appearing sooty black in mass. They were flask-shaped, with a short unicellular, light brown hyaline neck or beak. The body of the spore consisted of a varying number of cells separated by cross septa, the two bottom ones, in many cases, being further divided into smaller ones by vertical walls. The second cell from below had the largest diameter. The edges of the septa could be seen as rings encircling the spore. At the septa the spores were constricted. The wall was thick and echinulate except in the beak-cell which had thinner smooth wall. The spores, including the beak cell, consisted of 3-7 cells and varied between 10-40μ in length and 5-10μ in breadth, the beak cell alone measuring 3-7μ. The average measurement with the beak cell was 24μ × 7μ, the beak cell alone averaged 4·9μ in length, and the average number of cells including the beak was 4. In artificial cultures a secondary branch occasionally

came out from any of the cells of the spore which soon transformed into a secondary spore (Plate LXXI, fig. 2).

No marked difference was noticed between the sizes of spores from nature and those from the cultures on rice agar medium except that the average length of the former was 25μ in stead of 24μ as in the latter. Elliot [1917] noted that *Alternaria* spores produced on culture media tended to be smaller than spores borne on their food plants.

The extreme variability of the size and form of the spores can be judged from Tables III, IV, V and VI which give, for five hundred spores, the measurements of their length with the beak cell, their maximum breadth, the length of the beak cells alone, and the number of cells in each spore, as also the number of spores having the same measurement.

TABLE III.

Measurements of the length of spores.

Length including beak cells in microns	Number of spores out of 500 having this measurement
10-15	34
16-20	101
21-25	166
26-30	109
31-35	65
36-40	25

TABLE IV.

Measurements of the maximum breadth of spores.

Breadth in microns	Number of spores out of 500 having this measurement
5	55
6	181
7	80
8	19
9	125
10	40

TABLE V.

Measurements of the length of the beak cells.

Length in microns	Number of spores out of 500 having this measurement
3	92
4	219
5	51
6	60
7	78

TABLE VI.

Number of cells including the beak in each spore.

Number of cells	Number of spores out of 500 having as many cells
3	58
4	169
5	154
6	66
7	53

From the above tables the measurements of and the number of cells in the spores are as follows :—

Length including the beak cell—10-40μ
Majority—21-25μ
Average—24μ

Breadth maximum——. . 5-10μ
Majority-6μ
Average—7μ

Length of the beak cell——. 3-7μ
Majority—4μ
Average—4·9μ

Number of cells including the beak cell—3-7
Majority—4
Average—4

Method of spore formation :—The formation of spores was studied directly in young live colonies growing on a thin layer of rice agar medium. The extremity of

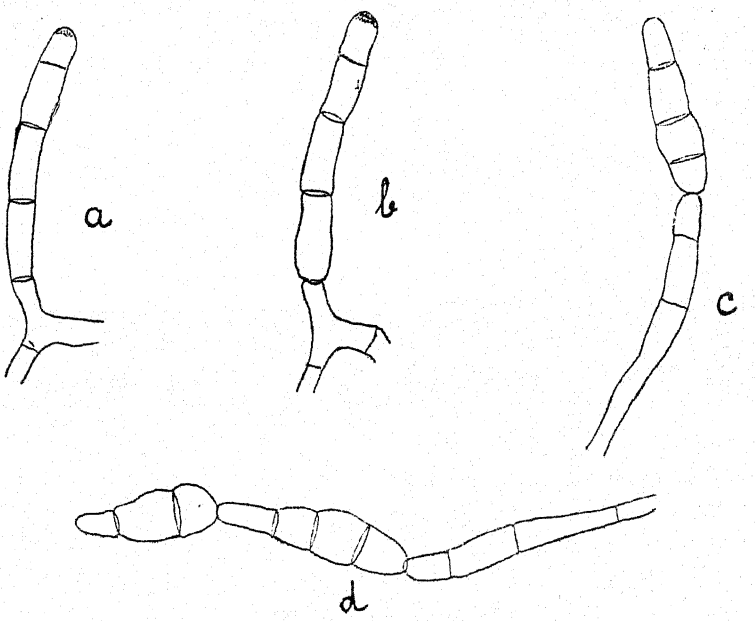

Fig. 1 (a-d)—Stages in the development of spores of *Alternaria* from the linseed plant. (Camera lucida drawings ×800.)

the main sporophore stopped elongating after a time, and became closely divided into three or four small cells by means of cross septa. The colour gradually deepened, except in the outward margin of the distal end cell which now appeared slightly refractile. This refractile portion was cut off by a wall and ultimately formed the beak cell. When the beak cell was being delimited, a constriction appeared, in the majority of cases, at the third septum, by the increase of diameter of the adjoining cells. Generally the second cell from the proximal end showed the greatest increase in size. Often vertical septa appeared in the two bottom cells. The beak cell, however, behaved differently. Instead of increasing in diameter it grew a little in length only. Ultimately the bottom cell more or less rounded off, the spore walls thickened and the colour deepened into dark brown in all the cells except in the beak, whose walls remained thin and the colour pale greenish brown. In this way a fully mature spore was produced.

Instances were noticed, especially when a big drop of water collected below the cover glass placed over the portion of the colony under examination, in which the refractile globules cut off from the tip of the main axis of the sporophore remained as such without undergoing further changes ; and below them regular spores with

beak cells were formed. In a few cases, the refractile globule gradually developed a thick wall, a partition appeared in its middle, transforming it into a spore without a beak cell. De Bary [1887] has figured a young sporophore of *Alternaria* on a mycelial filament submerged in water, in which a similar hyaline globule is shown.

Rands [1917] followed the spore formation in *Alternaria solani* and described it to have been produced from a bud, forming on the terminal cell of the sporophore. Jones' [1928] pictures of the different stages of the spore formation of *A. macrospora* indicate, that in this species a single, unicellular, terminal cell, abjointed from the sporophore, enlarged and developed within it a number of septa and thus grew into a septate spore. Thus, the spore formation of the *Alternaria* causing the blight of the linseed plant differed materially from that of *A. solani* and *A. macrospora*.

In this species, unlike the other two, a small apical portion of the sporophore gradually changed into a spore.

By the time the first spore was completed, a number of close septa appeared in the sporophore below, the endmost of the cells thus formed turning hyaline and refractile. No regular order of appearance of the cross septa was noticed, except that the one which delimited the spore was the first to appear. When these changes were taking place near the tip, the sporophore itself elongated from below. The formation of the second and the subsequent spores was exactly similar to the first one. In this way a chain of spores was produced. In the majority of cases, in the cultures on rice-agar medium, the catenula consisted of three spores. On the host plants it was not uncommon to find long chains containing six to eight spores.

Thus in this fungus the spores were terminal chlamydospores, produced in succession by acropetal fragmentation of the sporophore branches.

Germination of spores.—The spores germinated readily in tap water. It was rather difficult to wet the spores, due, no doubt, to the presence of spines on their surface, which formed air pockets within their meshes. The floating spores, being attracted by surface tension, tended to collect together in masses. In making a satisfactorily thick suspension the spore masses had to be stirred vigorously. But in doing so the sporophores, which were very brittle, broke up into small bits. The immature spores were not detached clean from the sporophores, but short pieces of the latter remained attached to their bases like stalks. The débri of sporophores in the suspension were removed by pipetting off the supernatant water, the wetted spores having settled to the bottom. Very soon after sinking to the bottom the spores became glued to the glass surface, so that a jet of water could not remove them easily. This might be due to gelatinization of the surface of the spores. No direct evidence of this gelatinization, by staining or mounting in Chinese ink [Blackman and Wilsford, 1916] could, however, be obtained. The beak cell stuck

faster to the glass surface, as could be evidenced from the fact that when a jet of water from a wash bottle was allowed to play on the spores, they seemed to be rooted by means of the beak cells on which they oscillated.

At 85° F. the spores germinated within four hours. They did not swell up appreciably in water before germination. The episporium burst at any point in any one or all the cells of the spore, the break gradually widened, and through it the protoplasm, enclosed within the endosporium, becoming increasingly turgid by imbibition of water, emerged in the form of a germ tube (Plate LXXI, fig. 1a, 1b.). The relation between the episporium, endosporium and the germ tube was clearly brought out by staining the slide with a very dilute gentian violet (one drop of concentrated gentian violet in 50 c.c. of water) (Plate LXXI, fig. 1d). The episporium was thick, dark brown cell-like structure, having minute spiny warts on the outer surface. The endosporium was very thin and colourless and appeared to line the episporium, and enclosed the protoplasm within it. In germination, the endosporium also elongated to form the wall of the germ tube. In this respect, this *Alternaria* agreed with *A. macrosporium* [Jones, 1928]. Jones used the terms intine and extine, but here endosporium and episporium of De Bary [1887] are retained, as being more expressive.

The spore was capable of sending out as many germ tubes as there were cells, but usually only two to three of the cells germinated. Butler [1918] observed the same fact in *A. brassicæ* on *sarson*. In some cases (Plate LXXI, fig. 1c) the beak cell also elongated into one. This happened through rupture at its extremity and never laterally as in *A. macrosporium* [Jones, 1928].

IV. Inoculations.

A large number of healthy cut branches of the linseed plants, containing buds, flowers and some young pods, were washed thoroughly with sterilized water to remove, as far as practicable, dust and foreign spores from their surface. They were divided into ten lots and each kept covered separately with a bell-jar over a dish of water. The cut ends of the branches were kept dipped in beakers of sterilized water. Six of them were inoculated by heavily sprinkling them with a thick suspension in sterilized water of spores from seven days old cultures. The suspension drops were seen collected at the bases of every leaf, flower and pod. The remaining four lots were kept as controls, having been sprinkled with sterilized water.

They were kept near a window in the laboratory, where the temperature ranged between 74°-85°F. during twenty-four hours. The experiments were started on March 2. Below is chronicled the summary of observations, from which it is clear

that the fungus *Alternaria* was responsible for the blight of the linseed plants in the field:

Inoculated branches:—March 4.—Two days after inoculation the bases of practically all the upper leaves had turned dark brown. The discolouration had spread down the stem, in some cases to nearly a centimeter. The number of buds and flowers which had blackened and collapsed was 7, 15, 8, 22, 25 and 8 respectively in the six lots.

March 6.—The number of affected buds and flowers rose to 32, 52, 37, 28, 60 and 19 respectively. Some of them were covered with a white mycelial growth, together with the infected portions of their stalk. Practically all the upper leaves, including portions of the stem adjoining them and the stalks of the affected buds had turned completely black.

March 8.—The number of diseased buds and flowers was now 48, 71, 43, 38, 82 and 30 respectively leaving 3, 2, 2 and 6 healthy ones in the second, fourth, fifth and sixth lots. In lot No. 6 there were a few fully formed pods which were also infected, the discolouration, however, remaining confined to the sepals and passing a short distance down the stem. Except a few lower leaves, the infection had spread over all the branches. The white mycelial growth could be seen covering all the young portions of the branches. With a hand lens crowded chains of spores could be seen growing out from the blackened stem and among the white growth. Hand sections of the former revealed the fungus hyphæ permeating through the tissues.

The bell-jars from three out of these six lots were removed and the following observations were noted:—

March 10.—In the uncovered specimens the white colour of the mycelial growth was completely masked and looked sooty black on account of numerous black spores produced on them. The spore chains were visible even to the naked eye. The spores were similar to those in the inoculating suspension.

In those, which still remained under bell jars, the production of spores was scanty; on the other hand, there was more of mycelial growth. This indicated that dry conditions favoured spore formation, a fact which was supported by field observations, stated before.

Small bits of the blackened stem and flower stalks were now used for reisolating the fungus. A few from each lot were cut out, their surface disinfected by dipping in mercuric chloride (1/1000 solution) for 15 minutes, and then repeatedly washed in sterilized water to remove the adhering mercuric chloride. Each bit was then

immediately dropped into a Petri dish containing rice-agar medium. Fourteen cultures were thus prepared. They were left in the room temperature (74°-85°F.). Except in three dishes which were accidentally contaminated, all the others developed colonies, which agreed in every respect with that of the original fungus. On the third day, chains of the same type of spores appeared in most of the cultures. Thus the fungus was successfully reisolated from the inoculated plants:—

Uninoculated controls.—The controls remained unchanged throughout the period of the experiments. Many of the leaf buds opened, but the petals of the flowers had shed leaving the dried-up stamens and pistils projecting out through the closed calyx. There were some fully formed pods in these controls. They remained unchanged except turning slightly brown.

With the spores obtained from the reisolated cultures, a second set of inoculations, with cut leaves and flowers, was carried out. Small drops of suspension of these spores were sown on the surface of a large number of leaves both mature and immature, and flowers, and all were kept in sterile moist chambers. After 48 hours, while the older leaves remained unchanged, the very young immature leaves developed minute brown spots under the infection drops which indicated that at these spots the infection had taken place. The controls, where only sterile water was used as infection drops, remained quite unchanged. In the flowers the infection was quicker and more easy to detect. The ovaries turned brown and soft, in fact all the floral parts rotted away and collapsed. Plate LXX, fig. 2 is a photograph of a style magnified twelve times which was inoculated, 48 hours before, by placing a loopful of the spore-suspension on the stigma. The fungus had passed down the style to more than half its length and the attacked portion was covered with a white fleecy mycelial growth. In the photograph, a few spore chains, especially on the left side of the stigma, is seen more clearly than the mycelium, which is rather indistinct.

V. Taxonomy.

The chief distinguishing feature between *Macrosporium* and *Alternaria*, both of which have practically the same type of spores, is the catenulæ or chains of spores, which the latter produces under favourable conditions. The fungus under study produces the chains, under all normal conditions, both in nature and artificial cultures. Hence there is no doubt that it belongs to the genus *Alternaria*. *Macrosporium commune* which attacks *Linum* [Lindau, 1910] is a different fungus from and cannot be mistaken for the present one.

Elliot [1917] made a detailed study of the *Alternaria* and *Macrosporium* and came to the conclusion that "All the species of *Macrosporium* in Sylloge Fungorum belong to *Alternaria*". He divided them into seven groups according to the shape of their spores. But later he recast them tentatively into six groups on the basis of the measurements of their spores. The beak cells were excluded in these measurements. These six groups are *A. tenuis*, *A. brassicae*, *A. herculea*, *A. cucumarina*, *A. sonchi* and *A. brassicae* var. *microspora*. But the present fungus with its spore sizes varying between 7—33 $\mu \times 5$—10μ without the beak cell, or the average of $24\mu \times 7\mu$ with the beak cell, cannot be placed in any of these groups except perhaps in the last one. *A. brassicae* var. *somniferum* f. *microspora* Brun. has got spores measuring $25\mu \times 7{\cdot}5\mu$ [Lindau, 1910]. It is a leaf parasite of the *Brassica oleracea*. But Elliot points out that the spores of *A. brassicae* var. *microspora* have seldom the longitudinal septa. In the present fungus they are quite frequent.

Young [1929] was of opinion that although the spore sizes constituted a fundamental basis for separating species, yet since the ranges in the spore sizes had been described very incompletely, strong consideration must be given to food plants in classifying these fungi. In his tabulation of the species none are mentioned parasitizing the plants of the order *Linaceæ*. To establish whether there is any relation of this fungus with *A. brassicæ* var. *somniferum f. microspora* Brun., which it approaches the nearest in morphological characters, or with any other *Alternaria*, an extensive cross inoculation and detailed study in artificial media are necessary. In the meantime this form had best be designated as a distinct species and named as *Alternaria lini*, sp. nov., with the following characters:—

Vegetative hyphæ.—Almost colourless, submerged, sparsely septate, with straight branches; average thickness 4μ.

Sporophores.—Pale olive; long, decumbent; alternately branched; septate; usually bursting through the epidermis singly or in groups of two or three, rarely through the stomata; average thickness $3{\cdot}5\mu$.

Spores.—Dark olive; 3-7 celled; longitudinal septa common; $10\text{-}40\mu \times 5\text{-}10\mu$ including the beak cell; the latter short, tapering, $3\text{-}7\mu$ long; catenulate; clavate; base obtuse; constricted at transverse septa; echinulate except in the beak cell.

Habitat.—Living stem, leaves and flowers of *Linum usitatissimum* L.; does not produce target-board spots or lesions with concentric rings, but the effect is a uniformly diffused withering and blackening of the affected parts.

VI. Method of infection.

The method of infection of the linseed plant by *A. lini* was studied by sowing thick watery suspension of spores on the surface of plucked leaves and petals,

placed in moist chambers, as was done in the second set of the inoculation experiments. As stated before old mature leaves could not be infected when spore-suspension was placed on their surface. Only small immature leaves, which could be easily infected thus, were therefore selected for this study. They were washed with sterilized water to remove dirt and foreign spores from their surface, and placed on glass slides in sterilized moist chambers. Small drops of spore suspension in sterile water were placed on them, on the upper surface in some and on the lower in others. They were incubated at 85° F. After 18 hours minute brown spots could be seen in all these leaves below the infection drops. Such portions of the leaves were cut out, fixed in Carnoy's fluid and microtomed to 5μ thick sections, which were then stained with iron-alum hæmatoxylin and counterstained with Orange G. in clove oil.

It was, however, found that the epidermis at the points of infection remained darkly stained, so much so that it was impossible to study the details of infection. Jones [1928] probably encountered the same difficulty in studying the penetration of *A. macrosporium* into the host tissue, for although he mentioned that the cuticle was ruptured, he did not explain how this was effected. His drawings also are not very clear on this point. To avoid this difficulty, the petals of fully formed flowers were used instead of the leaves. In the petals, the cuticle being thin, penetration was very quick and the dark stain below the infection hypha was not so very persistent. The inoculated petals were fixed six hours after sowing of the spores on them. It was found by trial that at this interval a large number of penetrations could be observed. Beyond this period the invading hyphæ progressed too far into the interior of the host tissue, as can be seen in Plate LXXI, fig. 5, which was drawn from the material fixed after eight hours.

It was noted during the study of their germination, that the spores became firmly fixed to the glass surface. On the leaf surface also they became similarly attached, so that a jet of water would not remove them. This was effected probably by means of the 'mucilage' produced on their surface when placed in water. There was no direct evidence of this mucilaginous sheath, but in permanent mounts stained with iron-alum hæmatoxylin minute dark granular spots and threads round the spore and connecting it with the substratum and with other spores and germ tubes, if they happened to be near it, could always be seen (Plate LXXI, figs. 6, 7), as in *Botrytis cinerea* [Blackman and Welsford, 1916], *Colletotrichum lindemuthianum* [Dey, 1919] and *C. glœosporioides* [Dey, 1933]. They were taken as the artifacts produced by the dehydrating agents on the mucilaginous sheath.

The germ tube became similarly fixed at its tip to the leaf surface by its 'mucilage'. Anchored in this way both at the spore and at the tip, the apical

growth of the germ tube caused it to curve up into a knee. The echinulate surface of the spore prevented it from slipping back, while its mucilaginous sheath kept it firmly appressed to the surface. Further growth of the germ tube in this position exerted a steadily increasing vertical pressure, which caused deep indentation in the cuticle at the point of contact, ultimately resulting in a rupture. The cuticle being in tension, it immediately contracted at the point of rupture, producing a hole wider than the diameter of the germ tube. The latter penetrated through this rupture and began to grow in the epidermal layer. In Plate LXXI, fig. 6, the upper margin of the cuticle is seen contracted away while its lower margin is bent inwards, owing to the pressure of the entering germ tube. There was no evidence of dissolution of the cuticle or of the underlying tissues by substances secreted by the germ tube prior to penetration.

All the germ tubes coming out from a spore could effect entrance through the cuticle in this manner. In Plate LXXI, fig. 6, two more germ tubes have penetrated, but their points of entrance were not visible in the section.

In a few cases the germ tubes were noticed to have passed over the stomata without effecting entrance. This was perhaps due to the fact, that either the spore or the germ tube failed to anchor itself firmly in the immediate neighbourhood of the stomata.

Thus, the penetration of the germ tube of *A. lini* into the petal was brought about by the rupture of the cuticle by mechanical pressure exerted on it by its apical growth. In this respect it agreed with *Botrytis cinerea*.[Blackman and Welsford, 1916], *Colletotrichum lindemuthianum* [Dey, 1919] and *C. glœosporioides* [Dey, 1933]. It, however, differed from these fungi in the absence of appressoria, the attachment being effected here by the 'mucilage' on the spores and germ tubes, and helped by the spines on the former.

Once the germ tube punctured through the cuticle, its progress was rapid. It branched and rebranched through the host tissue, killing it and causing it to dry up. In the immediate neighbourhood of the invading hyphæ in Plate LXXI, fig. 6, the cells had collapsed, while a halo or a clearer area round the tips of the hyphæ indicated dissolution of the cell contents, due probably to enzymatic action. In Plate LXXI, fig. 5, however, this dissolution was not visible, neither in a large number of cases examined. Thus, there was no conclusive evidence that the fungus exuded any poison which killed the host cells in advance.

Although the fungus failed to penetrate through the thick cuticle of mature leaves, when the spores were sown on their surface, yet in the inoculation experiments the infection occurred at the base of practically all the leaves, irrespective of whether they were young or old. It must have gained entrance in cases of old leaves through the tender axillary buds.

VII. Suggestions for control.

The study of the life-history of the causal organism thus indicates that the disease is one of the low-lying badly drained fields. Therefore, such fields where water is likely to remain stagnated after rains should be avoided and only well-drained high fields be used for this crop. Wet weather at the time of flowering favours spread of the disease. Hence, if the winter rains are early, before the plants come to flower, the damage is likely to be insignificant. The late varieties, therefore, should be preferable to the early ones, especially for the low-lying tracts. Spraying with a fungicide, such as bordeaux mixture, before flowering will, no doubt, prevent the spread of the disease, by killing the germ tubes emerging from the spores, but it is doubtful whether the additional cost and trouble, which spraying will involve, will justify the small benefit which will be derived from it.

VIII. Acknowledgments.

The writer expresses his thanks to his assistant Mr. B. S. Nigam for help in this work, to Messrs. Harbhajan Lal and T. S. Sabnis for supplying the yield figures of healthy and diseased plots at Gorakhpur and Cawnpore respectively, and to Dr. A. K. Mitra for helpful suggestions and correcting the manuscript.

IX. Summary.

An *Alternaria* blight of the linseed plant causes an appreciable loss in the water-logged fields in the United Provinces. It is particularly severe, if wet weather sets in at the time of flowering.

The symptoms of the blight and the morphological characters of the causal organism are described. Inoculations were successful and established the pathogenicity of the organism.

The causal organism is an *Alternaria*. The name *A. lini* is suggested for it.

The spores are produced in succession by acropetal fragmentation of the sporophores and are considered to be compound chlamydospores.

Infection readily takes place through immature organs of the host where the cuticle is thin.

The process of infection has been followed. The spores anchor themselves on the surface of the host by means of the mucilage into which their outer surface changes when they are wetted. The spines on the spores help in this anchoring. The tips of the germ tubes also are fixed by their mucilaginous envelope. Thus fixed at both ends further apical growth of the germ tubes causes the cuticle to rupture by mechanical pressure. The fungus gains entrance into the host tissue

through this puncture. In this respect it agrees with *Botrytis cinerea, Colletotrichum lindemuthianum* and *C. glœosporioides.*

There was no evidence that the fungus exuded any poison which killed the host cells in advance.

Suggestions are made for control. Well drained high fields only should be selected for linseed. Late varieties should be preferable for wet tracts. Use of a fungicide would not justify the cost and trouble it would involve.

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TYPES OF *SESAMUM INDICUM* D. C. IN THE PUNJAB.

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(With Plates LXXII-LXXIV.)

INTRODUCTION.

Til (*Sesamum indicum* D. C.) ranks next in importance to the Brassicæ oilseed crops as regards acreage in the Punjab, and on the average of five years ending 1931-32 it occupied an area of 130,773 acres annually. It is grown to a more or less extent in almost every district of the Punjab. It is, however, chiefly grown in the districts of Gurdaspur, Multan, Kangra, and Amritsar where it constitutes about 60 per cent. of its total area in the Province.

Til is grown mostly as a *barani* (rain-fed) crop in the *kharif* (summer) season, and is often sown alone or mixed with other crops particularly *mash* (*Phaseolus radiatus*), cotton (*Gossypium genus*), and *juar* (*Andropogon Sorghum*). It does well in medium loam soil, and thrives particularly well in the *sailaba* lands, *i.e.*, riverian tract, of some of the south-western districts of the Province where the soil is rich in plant food and is retentive of moisture. It must be remembered, however, that the crop is liable to suffer to a considerable extent if water is allowed to stagnate in the field. It is believed by many farmers that a variety which has been grown in a certain type of soil in a particular locality does not thrive in another district, even though the cultural and irrigation treatments are not materially different. This has been found to be the case with some of the types, *viz.*, Nos. 3, 15, 20, 28, and 29 obtained from Pusa, which, when grown at Gurdaspur and Lyallpur in the Punjab, produced very weak and sickly plants. Similarly the crop raised from seeds obtained from Kangra (a hilly district in the Punjab) failed completely when grown at Lyallpur. It has also been observed that the types which grow successfully in the *barani* areas of Gurdaspur, fail to give a satisfactory crop when grown under canal irrigation at Lyallpur. In some cases, however, it may be possible to grow the crop successfully by changing the

time of sowing or by modifying the method of cultivation. For example, by sowing the crop on ridges instead of on flat, to avoid damage by irrigation water, which is generally applied by the flooding system in the canal-irrigated areas, and by hoeing the fields properly to secure better aeration for the plants, it was possible to raise a fairly decent crop of certain varieties which ordinarily did not do well at Lyallpur.

The senior author during the period of post-graduate training in the Botanical Section at Pusa in 1928-29 had made a special study of the work then being carried on there on the classification of Sesamum crop of India, and on his return to the Punjab started the work of classification of Punjab Sesamum on almost similar lines as were being followed at Pusa. It appears that the collection under study at Pusa did not contain a sufficient number of representative samples from the Punjab. Kashi Ram [1932] refers to having received only one sample from the Punjab, *i.e.*, from Hoshiarpur, from which he isolated Pusa Type No. 6.

An examination of the Sesamum crop grown by the farmers in different parts of the Punjab shows it to be an admixture of many types, which evidently did not find their way to the collection made at Pusa for the work of classification. Therefore, in order to isolate and describe the various types of Sesamum met with in the Punjab, a large collection of samples from all the districts of the Province was made in 1929, through the various officials of the Agricultural and Revenue Departments. To this collection was added the material previously collected from important Sesamum growing districts of the Province, and which had been under observation since 1927. The pure types isolated from this collection are described in this paper.

Flowering and pollination.—The flowers are borne in racemes, singly or 2 or 3 together in the axils of the upper leaves. The flowers on the main shoot are generally borne from about the eighth pair of leaves upwards. When borne singly, the two rudimentary buds in the axil remain undeveloped. These solitary flowers open in acropetal succession. In the cases where two or three flower buds occur in the axil of a leaf, the central bud opens first and fairly long time before the lateral flower buds. The central flowers in this case also open in acropetal succession as a rule.

Our observations with regard to the relative position of anthers and stigmas agree with those of Howard, and A. R. Khan [1919]; but at Lyallpur we have observed certain deviations from the description given by these workers regarding the time of opening of flowers, etc. An account of our observations is, therefore, given below:—

The flowers start opening at about 7 a.m. and most of them are open by 8 a.m. though some may continue opening till 10 a.m. As the flower opens, the two hairy

lobes of the bifid stigma begin to separate and become receptive. The anthers also start bursting and liberate their pollen at the same time. The pedicel of an open flower bends down so that generally the mouth of the corolla faces obliquely downwards at about an angle of 60°. In this slanting position of the corolla the dehisced anthers and the receptive stigma lie grouped together on the side away from the lower lobe of the corolla. By 7-30 a.m. or 8 a.m. the stigmas are generally covered with pollen and as insects start visiting flowers just after 8 a.m. a great deal of self-pollination is to be expected.

The corollas are shed off about 15 to 20 hours after the opening of flowers. The stamens fall off with the corollas. The styles however remain sticking to the ovaries and are discernible the next morning as white, filamentous bodies. Later, however, their colour changes as they start withering and in about two days' time they are completely withered.

It may be noted that in few flowers the anthers remain aborted and are shed off without liberating any pollen. In such cases, therefore, cross-pollination may be expected which is mostly brought about by insects.

Four insects namely *Apis florea*, *Andrena ilerda*, *Ceratina sexmaculata* and *Trichometallae pollinosa* were the common visitors of sesamum flowers at Lyallpur. *Apis florea* (honey-bee) was found to be more after the pollen than nectar as it was often observed biting the anthers with its back turned towards the lower lobe of the corolla. It is possible that this insect may be largely responsible for whatever crossing takes place in the *til* crop. In addition, thrips have also been met with in the flowers and it is probable that they may also be agents in bringing about cross-pollination.

The extent ot natural crossing may be judged from the amount of splitting that occurred in the various cultures under study. On the average of 3 years' observations about 5 per cent. of the cultures showed a number of stray plants due to natural crossing.

However, in *til* crop self-pollination is easy and by bagging quite a good setting is obtained. Continuous bagging does not decrease the vigour of the progeny.

Characters used as a basis for classification.

Altogether 34 types of Punjab Sesamum have been isolated on the basis of easily recognisable morphological differences. The characters used are :—

(*a*) Number of flowers in each axil.
(*b*) Colour of seed.
(*c*) Colour of corolla.
(*d*) Amount of hairiness of stem and capsule.
(*e*) Colour of stem and capsule.

(*f*) Maturity.

(*g*) Arrangement of leaves on the main shoot.

Other characters such as differences in habit of growth, height of plants, size of flowers, fruits and seeds, shape of leaves, and colour of petioles have not been employed because most of these characters were found to be affected considerably by environment. A reference to these, however, has been made in the detailed description of the distinguishing characters of the types given further. Although most of the characters used by us in differentiating the types are the same as those used by Zaitzev [1924] in Turkestan or by Kashi Ram [1930] at Pusa, yet we have not found it possible to follow strictly any one of these workers, as some of the characters used by them did not exhibit any marked varietal differences among the types isolated by us. On the other hand we found certain other characters which appeared to be more suitable for this purpose, and we have, therefore, made the necessary changes. Zaitzev classified the Turkestan Sesamum into 41 types on the basis of the following characteristics :—

(1) Opposite or alternate arrangement of leaves on the main shoot.
(2) Number of flowers in the axil.
(3) Amount of hairiness of stem.
(4) Colour of seeds.
(5) Presence or absence of false partitions in the capsule.
(6) Shape of leaves on the lower parts of the stem.
(7) Colour of stem.
(8) Colour of corolla.
(9) General habit of growth and number of branches.

Kashi Ram used the following main characteristics :—

(1) Number of flowers in the axil.
(2) Colour of seed.
(3) Colour of corolla.
(4) Amount of hairs and markings on the corolla.
(5) Maturity.

He has, however, made use of such characters as colour of capsule and number of loculi in capsules in distinguishing a few types. It will thus be seen that for our work on the isolation of types of *Sesamum indicum* in the Punjab, we have tried to follow Kashi Ram with this difference that instead of using the character of amount of hairiness and markings on corolla we have preferred to use the amount of hairiness and colouring on stem and capsule for the reason that among these types we found much more marked constant differences with regard to this character, than what was found to be the case with regard to the amount of hairs and markings on corolla. All the types isolated by us have four loculed capsules. However,

PLATE LXXII.

SIX FLOWERS IN THE AXIL.

a. Two opposite leaves at a node; *b*. Three opposite leaves at a node.

in one plant of Type 25 in the year 1931 a six-loculed capsule was met with, and no such capsule was found in the year 1932. Therefore, the question of number of locules as a character for classification of Punjab Sesamum does not arise.

Differences in characters used for the isolation of types.

The characters will be discussed in the order in which we have made use of them in the isolation of types.

(*a*) *Number of flowers in each axil.*—According to Kashi Ram the flowers are solitary or 2-3 together in a leaf-axil. Zaitzev has also described one-flowered and three-flowered forms. Most of the types isolated by us have one flower in the axil with two rudimentary lateral buds, and the remainder have three flowers in the axil. The plants of all one-flowered types are as a rule much more branched than those of the three-flowered types. Sometimes among the three-flowered forms one or both of the lateral flowers do not form capsules and we therefore find only one or two capsules instead of three in the axil of certain leaves. This is usually observed in the lowest parts of the stem or in a crowded population of these varieties. Nevertheless, all the plants of such varieties as a rule possess the characteristic of having three flowers in each axil of their leaves. Therefore, for the purpose of classification we have grouped the various types into two classes, *viz.*:—

(*i*) Having one flower in each axil, for example Types 1-24.

(*ii*) Having three flowers in each axil, for example Types 25-34.

It may be noted that in one of the cultures studied we found a number of plants having six flowers in the axils of some of their leaves (Plate LXXII). Of these six flowers, however, only 3 to 5 usually formed well developed pods. The progeny of such six-flowered plants did not breed true to this character, but this particular culture has, during each of the past two years, been showing a number of plants exhibiting this characteristic of having six flowers in the axil. The factors concerned are being further looked into. The plants showing this character are as a rule single stemmed without any branches. It may also be noted that all the three-flowered types show an opposite arrangement of leaves on the main shoot except in the case of one type which resembles the one-flowered types in this respect as it has an alternate arrangement of leaves. In the few plants having six flowers in the axils of some of their leaves we found the opposite arrangement of leaves on the main shoot in all cases. In this arrangement of leaves, there are always two opposite leaves at each node (Plate LXXII, fig. *a*.), but one plant having 3 opposite leaves at a node (Plate LXXII, fig. *b*.) and six flowers in the axils of many of them was met with in the culture above referred to in the year 1931. The seed got from

this plant did not germinate at all and no more plants with three leaves at a node have been noticed so far in the progeny of this or any other culture.

(*b*) *Colour of seed.*—The seeds of different types vary greatly in their colour which ranges from almost white through various shades of brown and grey to deep black. Kashi Ram has described different colours by means of a coloured plate in his paper above referred to, and the differences observed by us among the various types agree more or less closely with his observations. We have, therefore, used almost the same terms as used by him as regards the colour of seeds of various types as follows:—

1. Almost white.
2. Dirty white.
3. Light brown.
4. Brown.
5. Dark brown.
6. Smoke grey.
7. Black.
8. Deep black.

He has also noted differences in the nature of seed-coats of different types and has described them as rough or smooth. Seeds of all the types isolated by us have smooth seed-coats.

(*c*) *Colour of corolla.*—Very perceptible differences have been observed with regard to the colour of corolla among different types, and we have strictly followed the same different shades of purple as given by Kashi Ram, *viz.*:—

1. Very pale purple or almost white.
2. White with a purple tinge.
3. Light purple.
4. Purple.
5. Deep purple.

It may, however, be noted that the colour differences referred to above are more conspicuous during the main flowering season of the plants concerned. The flowers formed towards the end of the growing season do not exhibit these differences to the same extent, but the colour differences among the types isolated are fairly distinguishable even at that stage.

(*d*) *Amount of hairiness of stems and capsules.*—The surfaces of stems and capsules of plants of the types isolated showed marked differences in the amount of hairiness, the hairs being as a rule multicellular. Although this character is liable to be considerably affected by the environment, yet among the types isolated and grown under varying environmental conditions for a number of years these differences showed sufficient stability to justify its being taken as

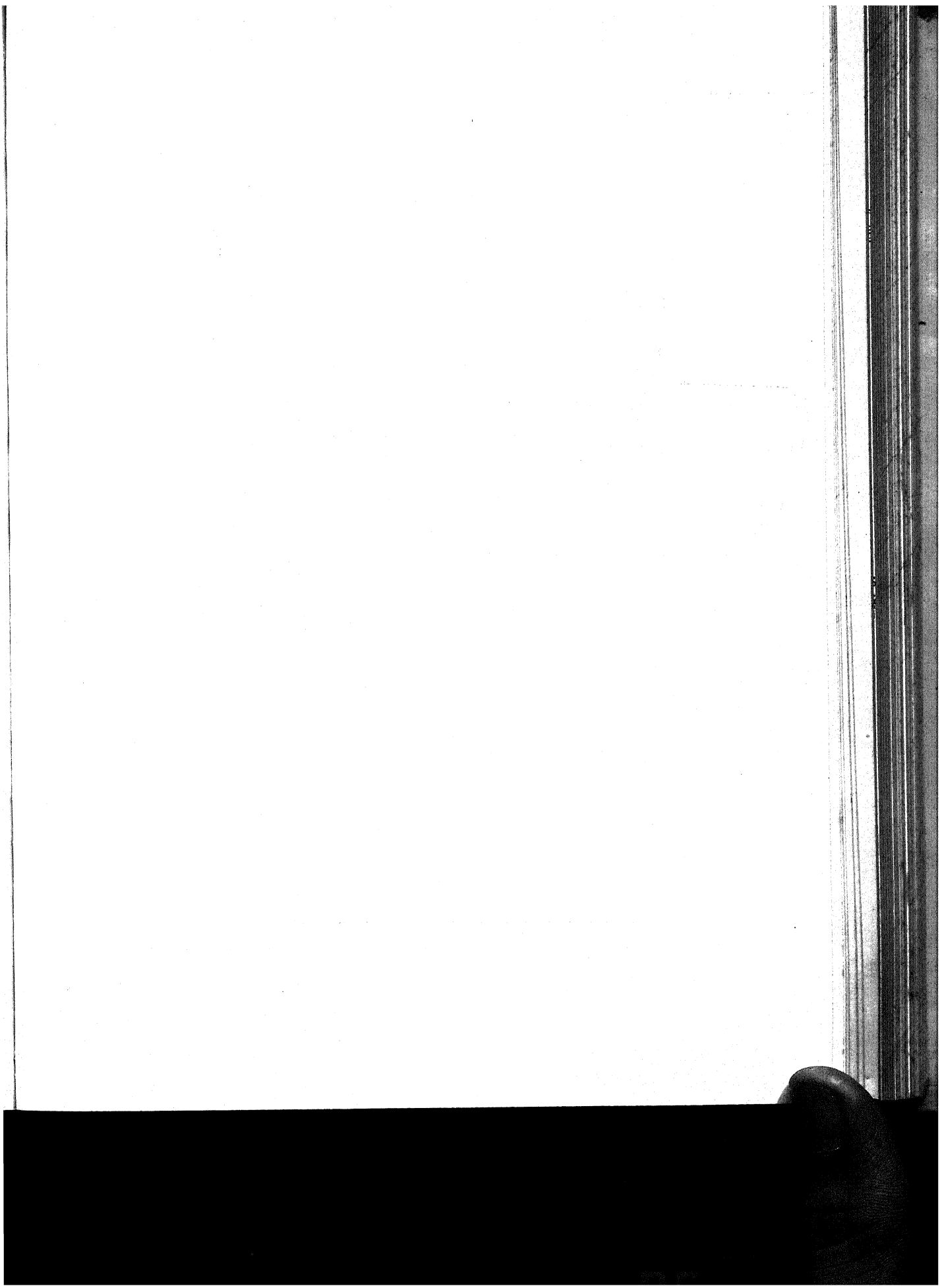
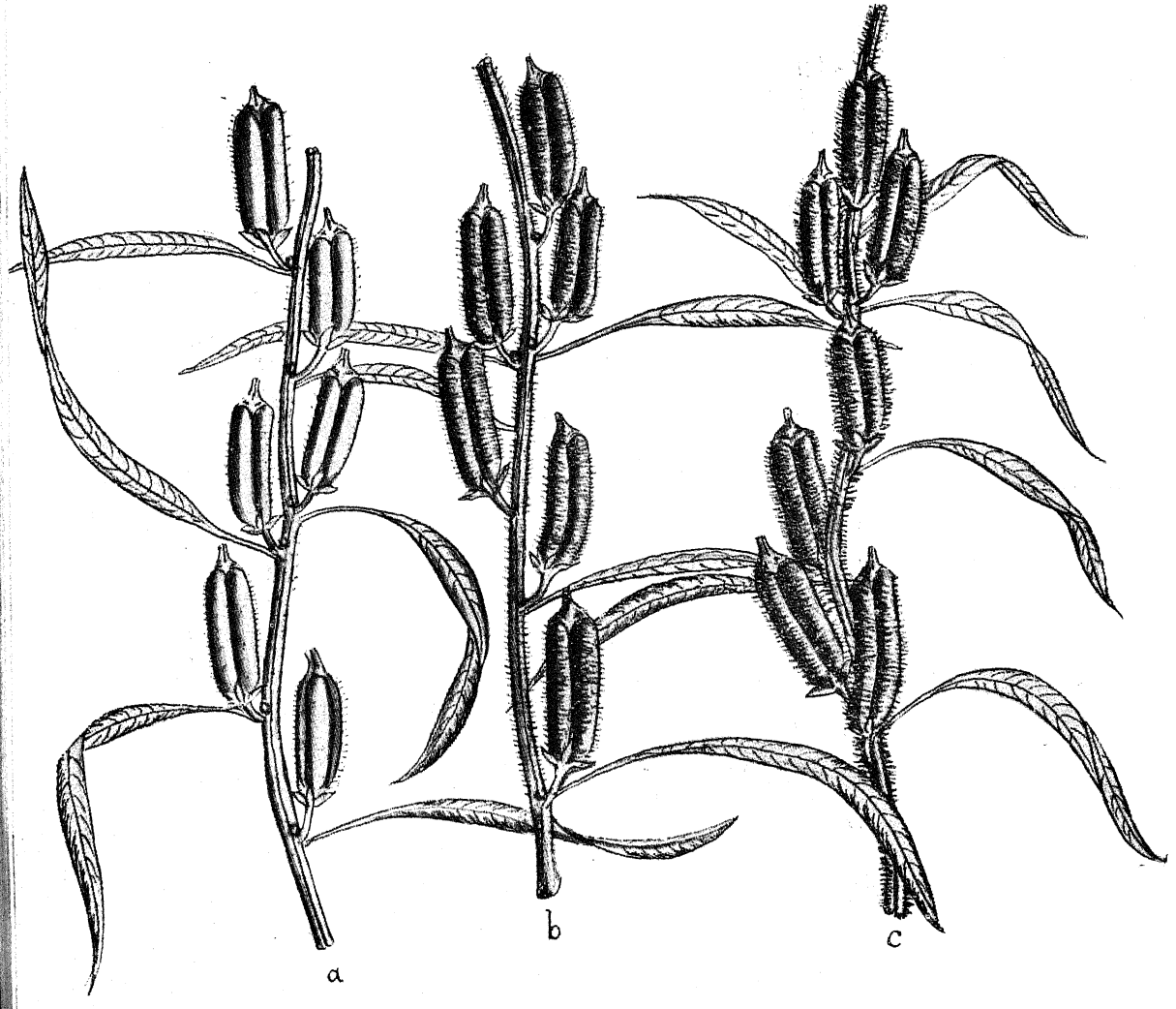


PLATE LXXIII.



THE RANGE IN HAIRINESS OF STEM AND CAPSULE.

a. Almost smooth; *b*. Hairy; *c*. Very profusely hairy.

a hereditary character of the types concerned. The surfaces of stems and capsules of certain types are very slightly hairy, or almost smooth, whereas in others they are hairy, or very profusely hairy (Plate LXXIII). This character appears to be of great economic importance as it has been observed that hairy types are comparatively more drought resistant, and much less liable to damage by birds, than the non-hairy types.

(*e*) *Colour of stems and capsules.*—Certain types show greenish or pale green stems and capsules with slight or no purple splashes on them, others show clearly marked purple bands or splashes, and still others show distinct purple colouration on almost the whole of their stems and capsules (Plate LXXIV). This colouration on stems and capsules is no doubt, like the colour of the corolla, a hereditary character. The intensity of colour of corolla is not, however, correlated with the intensity of colour of stem and capsule, as plants of certain types having purple corollas show very little or no purple colouration on their stems and capsules and *vice versâ*. The colour of stem however seems to be closely associated with that of capsules, so far as can be determined from the observations made on the types isolated by us.

(*f*) *Maturity.*—Plants of the types isolated have been described as early, medium, and late in maturity.

(*g*) *Opposite and alternate arrangement of leaves on the main shoot.*—This character has already been discussed and is of minor importance in the classification work.

KEY TO THE TYPES OF *Sesamum indicum* D. C.

One flower in each axil.
- Seeds almost white.
  - Corolla pale purple or almost white.
    - Stem and capsule almost smooth.
      - Capsules turn whitish near maturity . . . . . Type 1
- Seeds dirty white.
  - Corolla pale purple or almost white.
    - Stem and capsule almost smooth.
      - Capsules do not turn whitish near maturity . . . . Type 2
    - Stem and capsule hairy . . . . . Type 3
  - Corolla light purple.
    - Stem and capsule almost smooth.
      - Plants very early . . . . . Type 4
      - Plants medium in maturity . . . . . Type 5
    - Stem and capsule hairy . . . . . Type 6

Seeds light brown.
Corolla light purple.
Stem and capsule almost smooth.
Plants very early . . . . . Type 7

Plants very late . . . . . Type 8

Corolla purple.
Stem and capsule almost smooth . . . . . Type 9

Stem and capsule hairy . . . . . Type 10

Seeds brown.
Corolla white with purple tinge.
Stem and capsule very profusely hairy . . . . . Type 11

Corolla light purple.
Stem and capsule almost smooth and pale green with very few purple splashes . . . . . Type 12

Stem and capsule hairy and pale green . . . . . Type 13

Stem and capsule very hairy and pale green with few purple splashes . . . . . Type 14

Seeds dark brown.
Corolla light purple.
Stem and capsule almost smooth . . . . . Type 15

Corolla purple.
Stem and capsule almost smooth . . . . . Type 16

Seeds smoke grey.
Corolla light purple.
Stem and capsule hairy . . . . . Type 17

Seeds black.
Corolla light purple.
Stem and capsule almost smooth . . . . . Type 18

Corolla purple.
Stem and capsule almost smooth . . . . . Type 19

PURPLE COLOURED STEMS AND CAPSULES.

a. Almost smooth. *b*. Hairy.

Seeds deep black.
Corolla light purple.
Stem and capsule almost smooth, and green with a few purple splashes . . . . . Type 20
Stem and capsule almost smooth, and distinctly purple . . Type 21

Corolla purple.
Stem and capsule almost smooth with brownish splashes . . Type 22
Stem and capsule hairy and distinctly purple.
Plants comparatively early . . . . . Type 23
Plants comparatively late . . . . . Type 24

Three flowers in each axil.
Seeds dirty white.
Corolla white with purple tinge.
Stem and capsule almost smooth . . . . . Type 25

Corolla light purple.
Stem and capsule almost smooth . . . . . Type 26
Stem and capsule hairy . . . . . Type 27

Corolla purple.
Stem and capsule almost smooth.
Lower lobe of corolla deep purple . . . . . Type 28

Seeds light brown.
Corolla light purple.
Stem and capsule hairy . . . . . Type 29

Corolla purple.
Stem and capsule hairy . . . . . Type 30

Seeds black.
Corolla white with purple tinge.
Stem and capsule almost smoooth . . . . . Type 31

Corolla light purple.
Stem and capsule almost smooth.
Leaves on main shoot opposite . . . . . Type 32
Leaves on main shoot alternate . . . . . Type 33

Seeds deep black.
Corolla light purple.
Stem and capsule almost smooth . . . . . Type 34

Detailed distinguishing characteristics of the types.

Type 1.—Plants medium in maturity, somewhat open and profusely branched, medium in height (159 cm.); stem pale green with practically no purple splashes, almost smooth; leaves alternate, divided, petioles pale green; one flower in each axil; corolla almost white, large sized (4·2 cm. × 2·6 cm.), lower lobe almost white; capsules four loculed, almost smooth, pale green turning whitish near maturity, medium sized (3·0 cm. × 0·9 cm.); seeds almost white, small, smooth.

Type 2.—Plants medium in maturity, somewhat open and profusely branched, medium in height (153 cm.); stem pale green with practically no purple splashes, almost smooth; leaves alternate, divided, petioles pale green; one flower in each axil; corolla almost white, large sized (4·2 cm. × 2·6 cm.), lower lobe very light purple; capsules four loculed, almost smooth, green with very few purple splashes, medium sized (3·0 cm. × 0·8 cm.); seeds dirty white, medium sized, smooth.

Type 3.—Plants medium in maturity, moderately branched; dwarf (height 106 cm.); stem greenish, hairy; leaves alternate, divided, petioles purplish; one flower in each axil; corolla almost white, large sized (4·2 cm. × 2·7 cm.), lower lobe light purple; capsules four loculed, hairy, greenish, medium sized (3·0 cm. × 0·8 cm.); seeds dirty white, medium sized, smooth.

Type 4.—Plants very early, open and moderately branched, medium in height (156 cm.); stem green with very few purple splashes, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, medium sized (3·7 cm. × 2·0 cm.), lower lobe light purple; capsules four loculed, almost smooth, green with very few purple splashes, large sized (3·6 cm. × 0·9 cm.); seeds dirty white, rather small, smooth.

Type 5.—Plants medium in maturity, moderately branched, branches ascending, medium in height (151 cm.); stem pale green, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, small sized (3·5 cm. × 2·1 cm.), lower lobe light purple; capsules four loculed, almost smooth, pale green, small sized (2·5 cm. × 0·8 cm.); seeds dirty white, rather small, smooth.

Type 6.—Plants rather early, moderately branched, medium in height (146 cm.); stem green with purple bands and splashes, hairy; leaves alternate, divided, petioles purple; one flower in each axil; corolla light purple, medium sized (4·0 cm. × 2·7 cm.), lower lobe light purple; capsules four loculed, hairy, purplish, medium sized (2·7 cm. × 0·8 cm.); seeds dirty white, small, smooth.

Type 7.—Plants very early, moderately branched, medium in height (133 cm.); stem green with very few purple splashes, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, small sized (3·7 cm. × 1·9 cm.), lower lobe light purple, capsules four loculed, almost smooth; green

with very few purple splashes, big sized (3·6 cm. × 0·9 cm.); seeds light brown, medium sized, smooth.

Type 8.—Plants very late, moderately branched, tall (height 183 cm.); stem greenish with purple tinge, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, medium sized (4·0 cm. × 2·4 cm.), lower lobe light purple; capsules four loculed, almost smooth, pale green with purple splashes, medium sized (2·9 cm. × 0·9 cm.); seeds light brown, medium sized, smooth.

Type 9.—Plants medium in maturity, rather profusely branched, branches somewhat ascending, medium in height (154 cm.); stem pale green with a few purple splashes, almost smooth, leaves alternate, divided, petioles purplish; one flower in each axil; corolla purple, medium sized (4·0 cm. × 2·7 cm.), lower lobe purple; capsules four loculed, almost smooth, pale green with many purple splashes, medium sized (2·8 cm. × 0·8 cm.); seeds light brown, medium sized, smooth.

Type 10.—Plants medium in maturity, moderately branched, medium in height (152 cm.); stem pale green with purple bands and splashes, hairy; leaves alternate, divided, petioles more or less purple; one flower in each axil; corolla purple, medium sized (4·0 cm. × 2·4 cm.), lower lobe purple; capsule four loculed, hairy, palish green with purple splashes, medium sized (3·0 cm. × 0·9 cm.); seeds light brown, medium sized, smooth.

Type 11.—Plants medium in maturity, moderately branched, dwarf (height 130 cm.); stem green with very few purple splashes, very profusely hairy; leaves alternate, divided, petioles purplish; one flower in each axil; corolla white with purple tinge, large sized (4·3 cm.×2·5 cm.); lower lobe white with purple tinge; capsules four loculed, very profusely hairy, green with very few purple splashes, small sized (2·4 cm.×0·8 cm.); seeds brown, medium sized, smooth.

Type 12.—Plants rather early, open, and moderately branched, medium in height (156 cm.); stem pale green with very few purple splashes, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, medium sized (3·7 cm.×2·0 cm.), lower lobe light purple; capsules four loculed, almost smooth, pale green with very few purple splashes, large sized (3·6 cm.×0·9 cm.); seeds brown, rather small, smooth.

Type 13.—Plants medium in maturity, moderately branched, medium in height (160 cm.); stem pale green, hairy; leaves alternate, divided, petioles pale green; one flower in each axil; corolla light purple, medium sized (4·0 cm.×2·4 cm.), lower lobe light purple; capsules four loculed, hairy, pale green, rather small sized (2·5 cm.×0·8 cm.); seeds brown, medium sized, smooth.

Type 14.—Plants medium in maturity, moderately branched, branches ascending, medium in height (140 cm.); stem green with a few purple splashes, very

hairy; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, medium sized (4·0 cm.×2·4 cm.), lower lobe light purple; capsules four loculed, very hairy, pale green with a few purple splashes, small sized (2·4 cm. ×0·7 cm.); seeds brown, medium sized, smooth.

Type 15.—Plants medium in maturity, rather sparsely branched, branches ascending, medium in height (145 cm.); stem green with very few purple splashes, almost smooth; leaves alternate, divided, petioles light purple; one flower in each axil; corolla light purple, small sized (3·4 cm.×2·5 cm.), lower lobe light purple; capsules four loculed, almost smooth, green with very few purple splashes, medium sized (3·0 cm.×0·8 cm.); seeds dark brown, medium sized, smooth.

Type 16.—Plants medium in maturity, somewhat open and rather profusely branched, growth of main shoot restricted, tall (height 186 cm.); stem greenish with purple bands and splashes, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla purple, small sized (3·4 cm.×2·1 cm.), lower lobe purple; capsules four loculed, almost smooth, green with purple splashes, medium sized (2·6 cm.×0·8 cm.); seeds dark brown, medium sized, smooth.

Type 17.—Plants rather late, somewhat open and rather profusely branched, tall (height 194 cm.); stem greenish with a few purple splashes, hairy; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, large sized (4·1 cm.×2·4 cm.), lower lobe light purple; capsules four loculed, hairy, green with very few purple splashes, medium sized (2·6 cm.×0·8 cm.) having a blunt small point at the apex; seeds smoke grey, medium sized, smooth.

Type 18.—Plants medium in maturity, somewhat open and moderately branched, medium in height (171 cm.); stem greenish with a few purple splashes, almost smooth; leaves alternate, divided, petioles pale green with slight purplish tinge; one flower in each axil; corolla light purple, large sized (4·2 cm.×2·5 cm.), lower lobe light purple; capsules four loculed, almost smooth, greenish with a few purple splashes, medium sized (2·9 cm.×0·8 cm.); seeds black, medium sized, smooth.

Type 19.—Plants medium in maturity, moderately branched, tall (height 195 cm.); stem pale green with purple splashes, almost smooth; leaves alternate, divided, petioles light purple; one flower in each axil; corolla purple, small sized (3·2 cm.×2·1 cm.), lower lobe light purple; capsules four loculed, almost smooth, greenish with a few purple splashes, large sized (3·7 cm.×0·8 cm.); seeds black, medium sized, smooth.

Type 20.—Plants rather late in maturity, moderately branched, branches somewhat ascending, medium in height (172 cm.); stem greenish with a few purple splashes near maturity, almost smooth; leaves alternate, divided, petioles purplish; one flower in each axil; corolla light purple, small sized (3·0 cm.×1·9 cm.), lower

lobe light purple; capsules four loculed, almost smooth, green with a few purple splashes, medium sized (2·7 cm. × 0·8 cm.); seeds deep black, medium sized, smooth.

Type 21.—Plants late, somewhat open and moderately branched, tall (height 188 cm.); stem pale green in early stages and distinctly purple near maturity, almost smooth; leaves alternate, divided, petioles distinctly purple; one flower in each axil; corolla light purple, medium sized (3·8 cm.×2·2 cm.), lower lobe light purple; capsules four loculed, almost smooth, pale green in early stages and distinctly purple near maturity, medium sized (2·7 cm.×0·8 cm.); seeds deep black, medium sized, smooth.

Type 22.—Plants medium in maturity, open and profusely branched, medium in height (157 cm.); stem palish green with dull purple or brownish splashes, almost smooth; leaves alternate, divided, petioles with purplish tinge; one flower in each axil; corolla purple, medium sized (3·6 cm.×2·0 cm.), lower lobe light purple; capsules four loculed, almost smooth, greenish with a few purple or brownish splashes, small sized (2·5 cm.×0·8 cm.); seeds deep black, medium sized, smooth.

Type 23.—Plants comparatively earlier in maturity than those of Type 24, moderately branched, medium in height (161 cm.); stem pale green in early stages and distinctly purple near maturity, hairy; leaves alternate, divided, petioles purple; one flower in each axil, corolla purple, medium sized (3·8 cm.×2·2 cm.), lower lobe purple; capsules four loculed, hairy, pale green in early stages and distinctly purple near maturity, medium sized (2·7 cm.×0·8 cm.); seeds deep black, medium sized, smooth.

Type 24.—Plants comparatively later in maturity than those of Type 23, somewhat open and rather profusely branched, medium in height (161 cm.); stem pale green in early stages and distinctly purple near maturity, hairy; leaves alternate, divided, petioles purple; one flower in each axil; corolla purple, medium sized (3·8 cm.×2·2 cm.), lower lobe purple; capsules four loculed, hairy, pale green in early stages and distinctly purple near maturity, medium sized (2·7 cm.×0·8 cm.); seeds deep black, medium sized, smooth.

Type 25.—Plants medium in maturity, sparsely branched, branches somewhat ascending, medium in height (163 cm.); stem pale green with purple splashes, almost smooth; leaves opposite, divided, petioles purplish; three flowers in each axil; corolla white with purple tinge, large sized (4·3 cm.×2·5 cm.), lower lobe light purple; capsules four loculed, almost smooth, pale green with purple splashes, small sized (2·5 cm.×0·9 cm.); seeds dirty white, medium sized, smooth.

Type 26.—Plants medium in maturity, somewhat open and rather sparsely branched, medium in height (168 cm.); stem greenish with purple splashes, almost smooth; leaves opposite, divided, petioles purplish; three flowers in each axil;

corolla light purple, large sized (4·2 cm. × 2·4 cm.), lower lobe light purple ; capsules four loculed, almost smooth, greenish with purple splashes, rather large sized (3·1 cm. × 0·9 cm.) ; seeds dirty white, medium sized, smooth.

Type 27.—Plants medium in maturity, rather sparsely branched, dwarf (128 cm.) ; stem pale green with purple splashes, hairy ; leaves opposite, divided, petioles purplish ; three flowers in each axil ; corolla light purple, small sized (3·4 cm. × 2·0 cm.), lower lobe rather purple ; capsules four loculed, hairy, pale green with purple splashes, small sized (2·3 cm. × 0·9 cm.) ; seeds dirty white, medium sized, smooth.

Type 28.—Plants medium in maturity, sparsely branched, branches somewhat ascending, medium in height (158 cm.) ; stem pale green with purple splashes, almost smooth ; leaves opposite, divided, petioles purplish ; three flowers in each axil ; corolla purple, large sized (4·4 cm. × 2·6 cm.), lower lobe deep purple ; capsules four loculed, almost smooth, pale green with purple splashes, medium sized (2·8 cm. × 0·9 cm.) ; seeds dirty white, medium sized, smooth.

Type 29.—Plants medium in maturity, sparsely branched, branches somewhat ascending, dwarf (134 cm.) ; stem greenish with purple splashes, hairy ; leaves opposite, divided, petioles purplish ; three flowers in each axil ; corolla light purple, small sized (3·4 cm. × 2·0 cm.), lower lobe purple ; capsules four loculed, hairy, greenish with a few purple splashes, small sized (2·3 cm. × 0·9 cm.) ; seeds light brown, medium sized, smooth.

Type 30.—Plants medium in maturity, branches practically *nil*, medium in height (147 cm.) ; stem pale green with purple splashes, hairy ; leaves opposite, divided, petioles purplish tinged ; three flowers in each axil ; corolla purple, large sized (4·3 cm. × 2·6 cm.), lower lobe purple ; capsules four loculed, hairy, pale green with a few purple splashes, small sized (2·2 cm. × 0·8 cm.) ; seeds light brown, medium sized, smooth.

Type 31.—Plants medium in maturity, branches practically *nil*, dwarf (133 cm.) ; stem pale green with very few purple splashes, almost smooth ; leaves opposite, divided, petioles with a slight purple tinge ; three flowers in each axil ; corolla white with purple tinge, large sized (4·2 cm. × 2·4 cm.), lower lobe light purple ; capsules four loculed, almost smooth, green with few purple splashes, medium sized (3·0 cm. × 0·9 cm.) ; seeds black, medium sized, smooth.

Type 32.—Plants medium in maturity, rather moderately branched, medium sized (171 cm.) ; stem greenish with few purple splashes, almost smooth ; leaves opposite , divided, petioles pale green ; three flowers in each axil ; corolla light purple, large sized (4·2 cm. × 2·5 cm.) ; lower lobe light purple ; capsules four loculed, almost smooth, greenish with few purple splashes, medium sized (2·9 cm. × 0·8 cm.) ; seeds black, medium sized, smooth.

Type 33.—Plants medium in maturity, somewhat open and rather moderately branched, tall (193 cm.); stem pale green with practically no purple splashes, almost smooth; leaves alternate, divided, petioles purplish tinged; three flowers in each axil; corolla light purple, large sized (4·2 cm. × 2·4 cm.), lower lobe rather purple; capsules four loculed, almost smooth, pale green with few purple splashes, large sized (3·5 cm. × 0·9 cm.); seeds black, medium sized, smooth.

Type 34.—Plants medium in maturity, sparsely branched, branches somewhat ascending, tall (height 193 cm.); stem greenish with few purple splashes, almost smooth; leaves opposite, divided, petioles with a slight purplish tinge; three flowers in each axil; corolla light purple, small sized (3·2 cm. × 2·1 cm.), lower lobe light purple; capsules four loculed, almost smooth, greenish with few purple splashes, medium sized (2·7 cm. × 0·8 cm.); seeds deep black, medium sized, smooth.

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FUNGI FROM BOMBAY.

BY

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In September, 1932, Dr. B. N. Uppal, Plant Pathologist to the Government, Bombay, Poona, India, sent to the senior author for study, determination and description, 66 specimens of fungi. These were studied by the junior author under the supervision of the senior author. The determinations and also the descriptions of 8 new species are herewith presented. Two specimens were referred to Dr. W. G. Solheim, and one to Mr. Hubert A. Harris. Type specimens are deposited in the herbaria of the University of Illinois, Urbana, Ill., U. S. A., and the College of Agriculture, Poona.

PERONOSPORALES.

Bremia.

Bremia lactucæ Regel. on *Sonchus oleraceus* L.

PERISPORIALES.

Meliola.

Meliola carissæ Doidge on *Carissa carandas* L.

Irenopsis.

Irenopsis crotonis (Stevens and Tehon) Stevens on *Pavetta indica* L.

Chætothyrium.

Chætothyrium pongamiæ Harris, sp. nov.

Mycelium superficial, epiphyllous, brownish or olivaceous, branching loosely at right angles or the hyphæ aggregating to form prosenchymatous crusts, hyphal cells

cylindric or constricted at the septa, 6-20 μ long by 3-10 μ wide; perithecia globose, membranous, bearing 6-18 setæ, 80-175 μ in diameter; setæ dark brown, non-septate, occurring chiefly on the perithecia but frequently arising from the mycelium, 20-50 μ long and 4-8 μ wide; asciclavate to ovate, shortly pedicellate, aparaphysate, 8-spored, 32-50 μ by 15-28 μ; ascospores hyaline, clavate, 3-6 septate, 18-30 μ by 6-7 μ.

On *Pongamia glabra* Vent.

SPHÆRIALES.

Pleosphœria.

Pleosphœria citri Arn. on *Citrus medica* L.

Physalospora.

Physalospora pandani Stevens and Peirce, sp. nov.

Perithecia scattered, subepidermal, astromate, dark, globose, 135-220 μ in diameter by 100-150 μ high, with a short ostiolate protrusion; asci 8-spored, paraphysate, slender, 70-90 μ by 12-17 μ; spores elliptical 5-7 μ by 13-16 μ, hyaline to greenish. No pycnidia present. Mycelium hyaline to brownish.

On *Pandanus* spp.

Physalospora psidii Stevens and Peirce, sp. nov.

Perithecia scattered, astromate, buried, with a short protruding ostiolate beak, subglobose, 120-165 μ high, 270-345 μ in diameter; asci paraphysate, clavate, 8-spored, 72-100 μ by 26-33 μ, width uniform, base attenuate; spores elliptical, hyaline, 13-16 μ by 30-37 μ.

Conidiophores 10-13 μ long; macroconidia continuous, elliptical, hyaline, 5-8 μ by 12-15 μ; microconidia continuous, elliptical, hyaline, 2-3 μ by 5-7 μ; mycelium intercellular, hyaline to brown when old.

On *Psidium guajava* L.

MICROTHYRIALES.

Asterina.

Asterina saccardoana Theiss. on *Sideroxylon tomentosum* Roxb.

Asterina sphœrotheca Karst. and Roum. on *Vitex negundo* L.

Stigmatea.

Stigmatea piperis Rhem. on *Piper* spp.

DOTHIDEALES.

Phyllachora.

Phyllachora arthaxonis P. Henn. on *Arthraxon meeboldii* Stapf.

Phyllachora euphorbiaceæ Rhem. on *Euphorbia* spp.

PEZIZALES.

Pseudopeziza.

Pseudopeziza repanda (Fr.) Karst. on *Rubia cordifolia* L.

UREDINALES.

Puccinia.

Puccinia arthraxonis (P. Henn.) Sydow and Butl. on *Arthraxon meeboldii* Stapf.
Puccinia obtegens (Lk.) Tul. on *Gonicaulon glabrum* Cass.

Aecidium.

Aecidium eleagni-latifoliæ Petch on *Elæagnus latifolia* L.

Uredo.

Uredo uguressæ Petch on *Flacourtia* spp.

LYCOPERDALES.

Cyathus.

Cyathus dimorphus Cobb. on *Arachis hypogea* L.

PHOMALES.

Plenodomus.

Plenodomus inæqualis Sacc. and Trott. on *Pandanus* spp.

Asteroma.

Asteroma piperis Allesch. on *Piper* spp.

Diplodia.

Diplodia sansevieriæ Sydow on *Sansevieria* spp.
Diplodia macrostoma Lev. on *Sesbania ægyptiaca* Prain.

Septoria.

Septoria prosopidis Stevens and Peirce, sp. nov.

Pycnidia numerous, superficial, solitary, black, amphigenous and caulicole, 160-250 μ in diameter, convex to subglobose; ostiole circular to oval, slightly raised, 8-11 μ, conidia filiform, 3-septate, hyaline, 3 μ by 36 μ.

On *Prosopis juliflora* DC.

MELANCONIALES.

Glœosporium.

Glœosporium Raciborskii P. Henn. on *Mangifera indica* L.

Ramularia.

Ramularia nymphearum (Allesch.) Ramsbottom on *Nymphæa speciosa.*

Myxosporium.

Myxosporium phormii Speg. on *Pongamia glabra* Vent.
Myxosporium microsporum Cooke and Hark. on *Pyrus malus* L.

Pestalozzia.

Pestalozzia palmarum Cke. on *Borassus flabellifer* L.

MONILIALES.

Oidium.

Oidium carneum Cooke on *Sida humilis.*
Oidium oxalidis McAlp. on *Oxalis corniculata* L.
Oidium cococarpum Stevens and Peirce, sp. nov.

Effused to tufted, though not dense; mycelium scanty, branched, septate; sporophores suberect to erect, short, septate or continuous; spores 8-10μ by 6-8μ, ellipsoid, hyaline, in short chains.

On seed of *Cocos nucifera* L.

Botrytis.

Botrytis cinerea Pers. on *Tridax procumbens* L.

Chloridium.

Chloridium epiphyllum (Wallr.) Sacc. on *Calotropis gigantea* Br.

Alternaria.

Alternaria, Elliot's group 6 [1917]. on *Ficus bengalensis* L.
Alternaria, Elliot's group 3. on *Cassia fistula* L.

Cercospora.

Cercospora lythracearum Heald and Wolf on *Lagerstroemia parviflora* Roxb.
Cercospora ixorae Solheim, sp. nov.

Spots amphigenous, circular to angular, more or less vein-limited, tan to brown, darker below, 0·5 to 11 mm.; border definite, raised, narrow, same colour as spot or darker; mycelium internal, olivaceous to olive-brown, 2·5μ to 4μ; olive-brown in tubercle and up to 6·5μ; conidiophores hypophyllous, densely tufted, rupturing the epidermis, mostly simple but occasionally with alternate branches, straight to flexuous, with a more or less bulbose base which is 4-5μ in diameter, tuberculate (tubercle 50-70μ in diameter), subhyaline, 12-32μ by 3·5-4μ, continuous or 1-2 septate; conidial scars distinct, or indistinct, about 1·5μ; conidia cylindrical, subhyaline, 14-?μ by 2·5μ.

On *Ixora coccinea* L.

Corynespora.

Corynespora colebrookiae Solheim, sp. nov.

Spots amphigenous, circular to irregular, somewhat vein-limited, 1-4 mm., dark brown to reddish brown with light brown to whitish irregular centers above; light brown to olive-brown with lighter coloured centers below; border indefinite; mycelium internal, hyaline to brownish, brown in stromata, 1·5-5·5μ; conidiophores hypophyllous, moderately to fairly densely tufted, emerging through the stomata but protruding only a little above the surface, at times also produced internally from the sides of the tuberculate stroma, simple, light brown to brown, 10-30μ by 2-3·5μ, 1-septate; conidia cylindrical, catenulate, subhyaline to olivaceous, 15-30μ by 2-3·2μ, continuous, probably septate at times.

On *Colebrookia oppositifolia* Sm.

Illosporium.

Illosporium hibisci Stevens and Peirce, sp. nov.

Sporodochia sunken in wounded cortex, irregular, light grey to yellow, becoming black, 60-90μ in diameter; conidiophores brown to olivaceous, branched, 3μ thick; conidia hyaline, ellipsoid, 1μ or 3μ.

On *Hibiscus esculentum* L.

Exosporium.

Exosporium palmivorum Sacc. on *Borassus flabellifer* L.

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GROUNDNUT AS A ROTATION CROP WITH COTTON.

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(With one text-figure)

I. Introductory.

With the decrease in the annual rainfall, Berar of to-day is essentially a *kharif* crop tract and there are only a few isolated areas, such as the deep, fertile valleys of the Purna and Penganga rivers where a *rabi* crop can be successfully raised. The main *kharif* crops being cotton and *juar*, the choice of rotation has, until recently, been confined to these two crops. Unfortunately, both these are soil exhausting crops and it is obvious that on land where they are grown continuously the soil will, in time, become denuded of those elements of which the crops require most. That this has been the case in Berar is indicated by the diminished yield to be seen in many places. There has been a tendency even to grow cotton year after year on the same land. The period of high prices, during and a few years after the Great War, actually gave an impetus to this pernicious practice. Cotton began to occupy larger and still larger proportion of the cultivators' holdings, while the production of *juar* was reduced to the minimum required for domestic consumption. The result has been that the Berar soil over vast areas has become "cotton sick".

The problem which confronts the Berar cultivator to-day is that of a low yield of cotton from an impoverished soil on which cotton and *juar* have been raised year after year, regardless of any consideration of rational farming. The recent fall in the prices of agricultural produce has accentuated the position and the cotton growers at present are faced with probably the most difficult economic situation. But out of adversity a lesson might be learnt. For, whatever the outcome of the present world depression, it is certain that the cultivator in future must pay greater attention to every detail of his industry. There is greater need than ever to increase the production per acre as an important means of decreasing the cost of production. The possibility of increased returns through the use of new and

improved varieties of cotton will not alone solve the problem. To make a permanent success of cotton, it must be made to form one member in a rotation of crops and not the sole crop that is taken off the same ground year after year. Once the cultivator can be made to realise this and practise a definite plan of rotation, a very great step in advance will have been taken. In the following pages are described briefly some of the successful results achieved on the Government Experimental Farm, Akola, in experiments with rotations. It now remains to persuade the growers to make use of the results obtained.

II. Experimental.

In investigations relating to the causes of the low yield of cotton in Berar, the question of rotation of crops has ranked first and an increasing amount of work has been devoted to it. It was realised at the outset that each crop in the rotation must be such as will commend itself to the cultivator as being worth while to grow, either as food or as a money crop, and this limited the choice, in our experimental work, to groundnut and *juar*. Investigations were, therefore, commenced to study the influence, under a definite plan of rotation, of groundnut and *juar* on the outturns of cotton, and to discover, if possible, the best order and combination of these crops with cotton for securing the maximum profit per acre.

Rotation experiments were accordingly laid out in one-tenth-acre plots, replicated five times, on a uniform and level piece of land, typical of the black cotton soil of Berar. In the year before the commencement of the experiments, the land was cropped with *juar*. The whole area was uniformly manured with cattle-dung at the rate of 40 lbs. nitrogen per acre and all cultural treatments given before and after sowing, have been alike.

The various rotations under experiment are outlined below:—

1. Cotton followed by groundnut alternately;
2. Cotton, *juar* and then groundnut;
3. Cotton two years in succession, then *juar* and then groundnut;
4. Cotton two years in succession followed by *juar*;
5. Cotton followed by *juar* alternately;
6. Cotton year after year;
7. Cotton two years in succession followed by groundnut.

These experiments have already completed eight years and the results of the ninth year have just come in hand. Table I appended at the end gives the yields of the three crops, cotton, *juar* and groundnut, in pounds per acre, from 1923 to 1931, together with the average figures for the whole period. The last column of this table shows the percentage increase of the various rotations over rotation No. 6, with which all others are compared.

During the course of these experiments, many important observations have been recorded which throw some light on problems associated with cotton growing, but here it is proposed to confine attention only to the yield and financial aspects of the experiment.

III. Effect of different rotations on yield of cotton.

Fig. 1 below shows at a glance the effect, on the yield of cotton, of the various rotations outlined above.

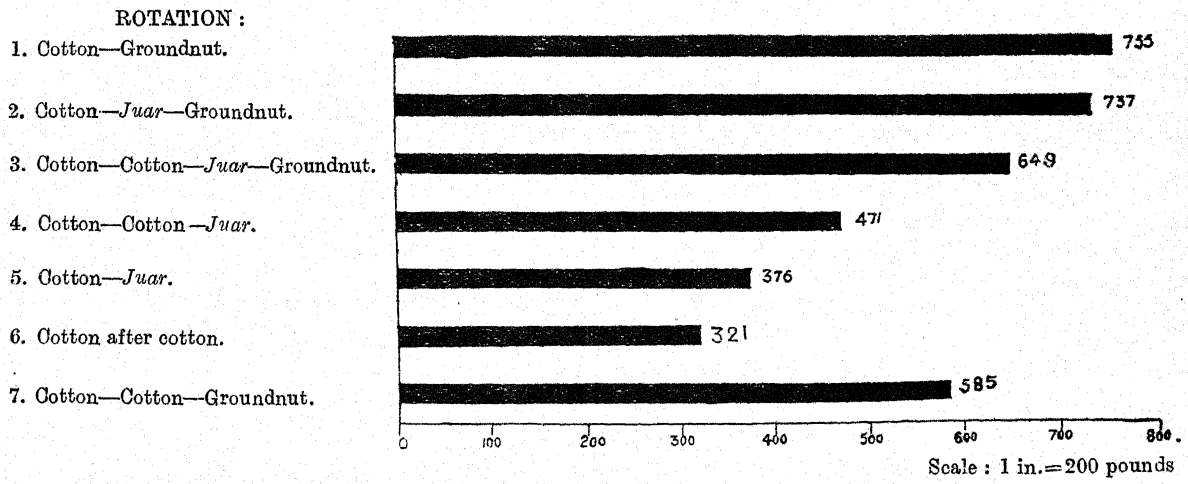

Fig. 1.—Average cotton yields under different rotations during the 8 years 1923-24 to 1930-31.

Rotation No. 1.—Of the seven rotations, this has given the highest average yield of *kapas* per acre during four complete cycles of rotation. Compared with No. 6 (cotton year after year) the increase obtained in No. 1 is 135 per cent. as will be seen by reference to Table 1.

Rotation No. 2.—From the point of view of yield this occupies the second place. The percentage increase in yield over the standard—No. 6—amounts to 130. If yield were the only consideration, the choice would no doubt fall on No. 1 (cotton and groundnut alternately). The difference in increase between No. 1 and No. 2 is, however, small, and in Berar where *juar* constitutes not only the principal food of the people but also provides '*karbi*' (dry fodder) for their cattle, the best rotation, as will be shown presently, is undoubtedly No. 2—a three-course rotation consisting of cotton, *juar* and groundnut.

Rotation No. 3.—This is a four-course rotation consisting of cotton, cotton, *juar* followed by groundnut. Compared with other rotations this stands third in order of merit. The percentage increase in yield over the standard—No. 6—being 102.

Rotations No. 4 and No. 5.—No. 4 is a three-course rotation of cotton, cotton followed by *juar*, while No. 5 is a two-course rotation consisting of cotton and *juar* alternately. These rotations represent roughly the type of rotation commonly practised in Berar, the number of years that cotton comes in the rotation being governed by the prices of cotton from year to year.

It is curious, however, that in spite of *juar* being a particularly exhaustive crop, both these rotations should give a higher yield of *kapas* than the standard, No. 6. But a closer examination shows that *juar* reacts favourably on the succeeding cotton crop by diminishing the incidence of fungoid and insect diseases which, in no small measure, reduce the ultimate yield of cotton.

Rotation No. 6.—Consisting as it does, of cotton year after year, this rotation has been introduced to serve as standard for purposes of comparison with other rotations. This is obviously the most irrational method of farming, a fact which is clearly borne out in our experiments by the continuous drop in yield from year to year, owing to the depletion of the soil and increase in the incidence of fungoid and other diseases.

The average yield obtained under this treatment amounts to 321 lbs. per acre, under conditions of deep cultivation and manuring as stated above. It is obvious that the yield in the cultivators' field where cotton is grown year after year must be much less.

Rotation No. 7.—This is a three-course rotation, consisting of cotton, cotton, followed by groundnut. At the present low prices of cotton and owing to the exclusion from it of *juar*, this rotation is hardly likely to find favour with the cultivator, although, in respect of yield, it stands fourth.

IV. Financial aspect.

In the introduction of a new method of farming, it is the financial gain more than anything else that appeals to the cultivator. Even if he is convinced of a higher monetary return at the end of the season, there is usually the difficulty of securing a little more money which the adoption of a new practice may involve. It is necessary, therefore, that the rotation recommended to him is such as brings him a maximum of profit at a minimum annual outlay. Tables III, IV and V give details of the financial side of the experiment. The term "gross profit" in these tables means a gain from which all items of expenditure, except rent and taxes, which vary considerably, have been deducted. It is realised that the value and profit per acre under the various treatments are affected by any marked variation in the price of the produce in particular years, but as the experiment has been carried out for a number of years, the results achieved can be considered reliable enough for all practical purposes.

A study of the financial tables referred to above unmistakably points to rotation No. 2 as being far and away the best that can be recommended to the cultivator. The average annual expenditure amounts to Rs. 45-4-0 (Table III) while the total value realised per acre is Rs. 128-6-0 ; so that, with a comparatively low cost of production, this rotation offers the largest gross profit per acre.

It is true that rotation No. 1 gives the highest yield of *kapas* per acre, but from the financial point of view, it cannot compare with No. 2. This is due to twofold reasons—firstly, the high cost of groundnut cultivation and secondly, the absence from it of *juar* which gives valuable and readily marketable fodder. It is in fact for this reason that rotation No. 5 gives a profit of Rs. 5-4-0 over rotation No. 1.

In column 5 of Table IV, the figures of profit per acre derived from cotton alone, under the different rotations are given. It will be seen that the maximum profit per acre is again obtained from the three-course rotation—cotton, *juar*, followed by groundnut.

The results of these experiments which are summarised in Table V indicate clearly that the three-course rotation of cotton, *juar* and groundnut is the most suitable one for the black cotton soil of Berar. There is little doubt that its extension will go a long way towards solving the problem of low yield of cotton in these provinces.

V. Reduction in cost of harvesting.

In our investigation on the economics of crop production, the question of reduction in the cost of production of agricultural produce always receives the closest attention. Hitherto, the chief obstacles in the way of extension of groundnut cultivation have been the high cost of lifting and scarcity of female labour at the time of harvest. It is not unusual for the lifting period of groundnuts to coincide with cotton picking and as the latter operation is relatively light and easy, it is difficult to get suitable labour at the right time, with the result that a great part of the crop is lost or damaged. Furthermore, the harvesting of a good crop of groundnuts requires some 18-21 female coolies per acre and the difficulty of obtaining labour for this purpose, even if the Berar cultivator were convinced of the advantage of a three-course rotation, including groundnut, will have to be solved. It has been possible, however, to devise a method of simultaneous lifting and ploughing with a special plough. This method was successfully used on the Akola Farm last year and effectively solved these difficulties, at the same time resulting in a saving of about Rs. 5 per acre. No loss of pods occurs and the land is at the same time ploughed up six months ahead of the next sowing season, thus giving in addition, the advantage of a cultivated fallow on which cotton can be sown early in the following season, just before the break of the monsoon. This

new plough has been designed on the basis of experience gained in lifting groundnut by means of a Sabul plough without the mould-board. The essential thing in lifting groundnut is that the pods should not get detached from the plant, broken or left under-ground. The pods, in our early types, cluster round the plant about 2½ to 4 ins. below the surface of the ground and the new lifter is designed to cut an inch or so below the zone of pod formation and push up the complete plant without loss of pods or foliage. With the mould-board fitted, it can be used as a common inversion plough suitable for working on local soils from November to April. An account of the working of this implement will be given elsewhere; here, it is proposed to confine attention only to the economic aspect of the question.

The cost of harvesting groundnut by the ordinary method of pulling by hand and ploughing up the land subsequently, is given below:—

	Rs.	A.	P.
2 ploughmen at As. 8 per day	1	0	0
1 boy for the pair in the fore	0	4	0
2 pairs of bullocks	3	0	0
Total	4	4	0
Work done by a team as above	½ acre per day		
	Rs.	A.	P.
∴ Cost per acre	8	8	0
Add wages of 21 female coolies (at As. 4 per day) required for lifting an acre by hand	5	4	0
Total cost of lifting and ploughing one acre	13	12	0

Compared with this, the method of simultaneous lifting and ploughing which is recommended to the Berar cultivator, results in a net saving of Rs. 5-6-8 as shown below:—

	Rs.	A.	P.
Labour—			
2 men at the plough working 9 hours per day at As. 8 each	1	0	0
1 boy for driving the pair in the fore	0	4	0
2 pairs of bullocks at Rs. 1-8	3	0	0
8 female coolies for picking uprooted plants, tying and stacking at As. 4 per head per day	2	0	0
Total	6	4	0
Work done by a team as above	0·75 acre		
	Rs.	A.	P.
∴ Cost of lifting and ploughing one acre	8	5	4

	Rs.	A.	P.
	13	12	0
	—8	5	4
Saving	5	6	8

This method is thus cheaper, employs less labour and gives the additional advantage of early ploughing before the land gets too hard. At the same time, all winter weeds are destroyed before seeding and the soil gets a longer period of exposure to the weathering agencies.

VI. Improved groundnut varieties.

In addition to the rotation experiments described above, a considerable amount of selection and hybridization work has also been carried out on this crop and it has been possible to isolate pure lines of several nuts. All these types are of the erect habit which seems more suitable for local conditions than the spreading. Some of these have proved very promising but those of Small Japan, Spanish peanut and a large-podded variety, known as AK-10, are most in demand. The latter has big nuts, is easy to harvest and possesses the capacity for high yield, which more than compensates the low yield of oil and the consequent low price which it fetches in the market. In the comparative tests at the Akola Farm, it has consistently given the highest outturn—2,260 lbs., per acre, which is nearly 100 per cent. more than the Small Japan and Spanish peanut types. This variety can be recommended as an excellent eating nut.

Recently a new strain of Spanish peanut, known as AK 12-24, has been evolved. This strain possesses bigger pods, like those of Small Japan, with which it also rivals in respect of maturity and oil-content. In cropping power, it is superior to both Spanish peanut and Small Japan and is comparatively easy to harvest.

VII. Acknowledgment.

The investigations outlined above are partly financed by the Indian Central Cotton Committee to which our thanks are due.

TABLE I.

Outturn of crops per acre during the past eight years in the rotation experiments at the Government Experimental Farm, Akola.

Serial No. of plots	Rotation	Outturn per acre in pounds								Average yield of *kapas* for the past years excluding 1st year in cases where advantage of rotation not valuable	Average yield of cotton stated as percentage of standard, No. 6 rotation	Percentage increase in yield in various treatments over No. 6
		1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31			
1	Cotton and groundnut alternately.	*Kapas*=318	Pods=1590 Tops=1258	*Kapas*=506	Pods=1000 Tops= 890	*Kapas*=758	Pods=2300 Tops=1236	*Kapas*=1002	Pods=1372 Tops=2202	755	235·0	+135
2	Cotton, then *juar* and then groundnut.	..	*Kapas*=554	Grain=1566 *Karbi*=5484	Pods=1190 Tops= 930	*Kapas*=900	Grain=1308 *Karbi*=10396	Pods=1598 Tops= 950	*Kapas*=756	737	230·0	+130
3	Cotton, cotton *juar* then groundnut.	..	..	*Kapas*=438	*Kapas*=323	Grain=1680 *Karbi*=11122	Pods=1950 Tops=1142	.. *Kapas*=1220	.. *Kapas*=614	649	202 0	+102
4	Cotton, cotton then *juar*.	..	..	*Kapas*=336	*Kapas*=372	Grain=1440 *Karbi*=8878	*Kapas*=370	*Kapas*= 806	Grain=1446 *Karbi*=10160	471	147·0	+47
5	Cotton and *juar* alternately.	..	*Kapas*=374	Grain=1910 *Karbi*=5448	*Kapas*=390	Grain=1258 *Karbi*=6244	*Kapas*=420	Grain=1546 *Karbi*=7940	*Kapas*=318	376	117·0	+17
6	Cotton after cotton.	*Kapas*=238	*Kapas*=254	*Kapas*=190	*Kapas*=228	*Kapas*=554	*Kapas*=252	*Kapas*=432	*Kapas*=418	321	100	..
7	Cotton, cotton then groundnut.	..	..	*Kapas*=410	*Kapas*=422	Pods=2340 Tops=1432	*Kapas*=902	*Kapas*=430	Pods=1706 Tops=2882	585	182	+82

TABLE II.

Outturn of cotton per acre stated as percentage of standard—No. 6 plot—from 1923-24 to 1930-31.

Plot No.	Rotation	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Average of eight years
1	Cotton, groundnut alternately.	*Kapas* = 318 *134*	..	*Kapas* = 506 *266*	..	*Kapas* = 758 *137*	..	*Kapas* = 1002 *232*	..	755 235
2	Cotton, *juar*, groundnut.	..	*Kapas* = 554 *218*	..	..	*Kapas* = 900 *162*	..	..	*Kapas* = 756 *181*	737 230
3	Cotton, cotton, *juar*, groundnut.	..	..	*Kapas* = 438 *231*	*Kapas* = 323 *142*	..	..	*Kapas* = 1200 *278*	*Kapas* = 614 *147*	649 202
4	Cotton, cotton, *juar*.	..	..	*Kapas* = 336 *177*	*Kapas* = 372 *163*	..	*Kapas* = 370 *147*	*Kapas* = 806 *187*	..	471 147
5	Cotton, *juar* alternately.	..	*Kapas* = 374 *147*	..	*Kapas* = 390 *171*	..	*Kapas* = 420 *167*	..	*Kapas* = 318 *76*	376 117
6	Cotton after cotton.	*Kapas* = 238 *100*	*Kapas* = 254 *100*	*Kapas* = 190 *100*	*Kapas* = 228 *100*	*Kapas* = 554 *100*	*Kapas* = 252 *100*	*Kapas* = 432 *100*	*Kapas* = 418 *100*	321 100
7	Cotton, cotton, groundnut.	..	..	*Kapas* = 410 *216*	*Kapas* = 422 *185*	..	*Kapas* = 902 *358*	*Kapas* = 430 *100*	..	585 182

TABLE III.

Annual and average value of all kinds of produce, cost of production and gross profit per acre together with the averages stated as percentages of standard treatment, No. 6, in the rotation experiment. Series "A" of the Government Experimental Farm, Akola, from the year 1923-24 to 1930-31.

Rotation	Total value realised per acre								Average value per acre for all cycles	
	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Amount	Percentage of standard, No. 6
	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	
1 Cotton, groundnut alternately.	79 2 0	142 15 0	73 4 0	94 11 0	113 12 0	171 7 0	96 8 0	75 0 0	195 13 0	242
2 Cotton, *juar*, then groundnut.	—	119 12 0	119 12 0	110 2 0	134 14 0	166 5 0	119 4 0	*43 6 0	128 6 0	294
3 Cotton, cotton, *juar*, then groundnut.	—	—	63 7 0	35 5 0	183 11 0	145 11 0	*117 8 0	*35 4 0	107 1 0	245
4 Cotton, cotton, then *juar*.	—	—	48 10 0	41 1 0	150 15 0	52 12 0	77 10 0	69 13 0	73 8 0	168
5 Cotton, *juar*, alternately.	—	80 14 0	133 15 0	43 4 0	116 12 0	59 5 0	140 1 0	18 4 0	95 11 0	219
6 Cotton after cotton .	54 3 0	59 4 0	27 8 0	25 2 0	81 11 0	35 9 0	41 10 0	24 0 0	43 10 0	100
7 Cotton, cotton, then groundnut.	—	—	59 6 0	46 9 0	199 14 0	127 7 0	41 7 0	94 14 0	94 13 0	217

Rotation	Total cost of production per acre								Average cost of production per acre	
	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Amount	Percentage of standard, No. 6
	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	Rs. a. p.	
1 Cotton, groundnut alternately.	33 2 0	76 3 0	33 4 0	66 12 0	37 5 0	71 0 0	51 14 0	55 15 0	53 3 0	147
2 Cotton, *juar*, then groundnut.	—	34 11 0	34 0 0	67 9 0	38 11 0	41 14 0	54 13 0	*39 9 0	45 4 0	128
3 Cot on, cotton, *juar*, then groundnut.	—	—	32 3 0	41 11 0	40 7 0	64 13 0	*55 4 0	*40 7 0	44 13 0	124
4 Cotton, cotton, then *juar*.	—	—	32 3 0	42 5 0	39 8 0	36 13 0	51 5 0	39 8 0	40 4 0	112
5 Cotton, *juar*, alternately.	—	33 12 0	34 12 0	41 9 0	39 5 0	37 10 0	39 11 0	*36 8 0	37 13 0	105
6 Cotton after cotton .	—	25 9 0	30 5 0	40 10 0	37 8 0	34 11 0	35 7 0	38 8 0	36 1 0	100
7 Cotton, cotton, then groundnut.	—	—	33 5 0	42 14 0	66 10 0	43 9 0	45 7 0	57 15 0	48 5 0	134

*Omitted in calculation as they did not cover a complete cycle period.

TABLE III—*contd.*

Rotation	Gross profit per acre								Average gross profit per acre
	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	
	Rs. a.	Rs. a.	Rs. a.	Rs. a.	Rs. a.	Rs. a.	Rs. a.	Rs. a.	Rs. a.
1 Cotton, groundnut alternately.	46 0	66 12	40 0	27 15	76 7	100 7	44 10	19 1	52 10
2 Cotton, *juar*, then groundnut.	..	85 1	85 8	42 9	96 3	124 7	64 11	3 12*	83 2
3 Cotton, cotton, *juar* then groundnut.	..	..	31 4	6 6	143 4	80 14	62 4*	5 3*	62 4
4 Cotton, cotton, then *juar*.	..	..	16 7	1 4	111 7	15 15	26 5	30 5	33 14
5 Cotton, *juar* alternately.	..	47 2	99 3	1 7	77 7	21 11	110 6	18 4*	57 14
6 Cotton after cotton	..	33 11	—2 13	15 8	44 3	0 14	3 13	—14 8	7 9
7 Cotton, cotton, then groundnut.	..	..	26 0	3 11	133 4	83 14	4 0	36 12	46 8

* Omitted in calculation as they did not cover a complete cycle period.

TABLE III-A.

Actual prices of the different crops in the rotations in different seasons.

Year	Price of *kapas* per khandi of 784 lbs.	Price of groundnut per palla of 246 lbs.	Price of *juar* grain per khandi of ten bags (2,146½ lbs.)
	Rs. a. p.	Rs. a. p.	Rs. a. p.
1930-1931	55 0 0	9 8 0	45 0 0
1929-1930	75 8 0	15 8 0	83 8 0
1928-1929	110 12 0	15 8 0	101 8 0
1927-1928	117 8 0	18 0 0	86 12 0
1926-1927	86 8 0	19 0 0	108 8 0
1925-1926	113 8 0	18 0 0	84 0 0
1924-1925	169 8 0	18 3 6	101 0 0
1923-1924 . . .	195 0 0	21 15 6	96 0 0

TABLE IV.

Average value, cost of production and gross profit per acre for cotton crop only, calculated at rates at which cotton was actually sold during the years 1923-24 to 1930-31.

Serial No.	Rotation	Average value of cotton per acre	Average cost of production per acre	Average gross profit per acre
		Rs. a. p.	Rs. a. p.	Rs. a. p.
1	Cotton, groundnut alternately . . .	94 8 0	40 13 0	53 11 0
2	Cotton, *juar*, then groundnut . . .	99 5 0	37 10 0	61 11 0
3	Cotton, cotton, *juar*, then groundnut . .	62 13 0	42 6 0	20 7 0
4	Cotton, cotton, then *juar*	55 0 0	40 11 0	14 5 0
5	Cotton, *juar* alternately	50 7 0	37 6 0	13 1 0
6	Cotton after cotton	43 10 0	36 1 0	7 9 0
7	Cotton, cotton, then groundnut . . .	71 7 0	43 15 0	27 8 0

TABLE V.

Average value, cost of production and gross profit per acre for complete cycle or cycles of rotations for all kinds of crops grown in rotation experiments during the years 1923-24 to 1930-31.

Serial No.	Rotation	Average value per acre		Average cost of production per acre		Average gross profit per acre
		Amount	Percentage No. 6	Amount	Percentage No. 6	
		Rs. a. p.		Rs. a. p.		Rs. a. p.
1	Cotton, groundnut alternately.	105 13 0	242	53 3 0	147	52 10 0
2	Cotton, *juar*, then groundnut	128 6 0	294	45 4 0	128	83 2 0
3	Cotton, cotton, *juar*, then groundnut.	107 1 0	245	44 13 0	124	62 4 0
4	Cotton, cotton, then *juar* .	73 8 0	168	40 4 0	112	33 4 0
5	Cotton, *juar* alternately .	95 11 0	217	37 13 0	105	57 14 0
6	Cotton after cotton . .	43 10 0	100	36 1 0	100	7 9 0
7	Cotton, cotton, then groundnut	94 13 0	217	48 5 0	134	46 8 0

TABLE VI.

Yields of each of the five replicates of rotational experiments from 1923-24 to 1930-31.

Replications	Treatment No. 1 — Outturn per 1/10 acre plot in lbs.								Treatment No. 2 — Outturn per 1/10 acre plot in lbs.						
	Cotton 1923-24	Groundnut 1924-25	Cotton 1925-26	Groundnut 1926-27	Cotton 1927-28	Groundnut 1928-29	Cotton 1929-30	Groundnut 1930-31	Cotton 1924-25	*Juar* 1925-26	Groundnut 1926-27	Cotton 1927-28	*Juar* 1928-29	Groundnut 1929-30	Cotton 1930-31
A—1	36	159 Nuts 101 Tops	36	93 Nuts 84 Tops	59	217 Nuts 119 Tops	74	133 Nuts 184 Tops	66	149 Grain 469 *Karbi*	134 Nuts 103 Tops	85	128 Grain 1,133 *Karbi*	170 Nuts 88 Tops	64
A—2	35	156 Nuts 122 Tops	69	.. ..	102	214 Nuts 138 Tops	130	134 Nuts 224 Tops	55	149 Grain 567 *Karbi*	.. ..	90	145 Grain 969 *Karbi*	145 Nuts 93 Tops	83
A—3	36	156 Nuts 111 Tops	37	109 Nuts 93 Tops	42	259 Nuts 134 Tops	65	134 Nuts 180 Tops	57	133 Grain 445 *Karbi*	107 Nuts 80 Tops	84	134 Grain 959 *Karbi*	155 Nuts 89 Tops	66
A—4	29	124 Nuts 122 Tops	58	.. ..	90	245 Nuts 120 Tops	127	131 Nuts 263 Tops	39	129 Grain 555 *Karbi*	.. ..	100	130 Grain 902 *Karbi*	128 Nuts 91 Tops	89
A—5	23	200 Nuts 173 Tops	53	97 Nuts 91 Tops	86	215 Nuts 152 Tops	105	154 Nuts 250 Tops	60	223 Grain 706 *Karbi*	116 Nuts 97 Tops	91	117 Grain 1,235 *Karbi*	201 Nuts 114 Tops	76
Average per acre outturn in lbs.	318	1,590 Nuts 1,258 Tops	506	1,000 Nuts 890 Tops	758	2,300 Nuts 1,326 Tops	1,002	1,372 Nuts 2,202 Tops	554	1,566 Grain 5,484 *Karbi*	1,190 Nuts 930 Tops	900	1,308 Grain 10,396 *Karbi*	1,598 Nuts 950 Tops	756

Treatments—

1. Cotton after groundnut, alternating from year to year
2. Cotton, then juar, then groundnut
3. Cotton for two years running, then *juar* and then groundnut
4. Cotton for two years running and then *juar*
5. Cotton one year followed by *juar* the next

Treatments—

6. Cotton followed by cotton year after year
7. Cotton for two years running and then groundnut
8. Cotton followed by cotton year after year
   This is the same as No. 6 started in 1925-26 to corroborate the results of No. 6

NOTE.—Blanks in yield columns under 1926-27 are due to failure owing to excessive water logging

TABLE VI—*contd.*

Yields of each of the five replicates of rotational experiments from 1923-24 to 1930-31—contd.

Replications	Treatment No. 3 Outturn per 1/16 acre plot in lbs.						Treatment No. 4 Outturn per 1/16 acre plot in lbs.					
	Cotton 1925-26	Cotton 1926-27	*Juar* 1927-28	Groundnut 1928-29	Cotton 1929-30	Cotton 1930-31	Cotton 1925-26	Cotton 1926-27	*Juar* 1927-28	Cotton 1928-29	Cotton 1929-30	*Juar* 1930-31
A—1 . .	42	38	189 Grain 1,177 *Karbi*	222 Nuts 134 Tops	108	63	35	55	165 Grain 1,022 *Karbi*	31	65	174 Grain 1,251 *Karbi*
A—2 . .	49	..	136 Grain 1,016 *Karbi*	192 Nuts 95 Tops	136	49	35	27	127 Grain 1,008 *Karbi*	36	77	144 Grain 912 *Karbi*
A—3 . .	44	32	179 Grain 1,096 *Karbi*	160 Nuts 111 Tops	112	63	34	45	167 Grain 804 *Karbi*	39	83	157 Grain 1,075 *Karbi*
A—4 . .	52	..	145 Grain 1,004 *Karbi*	179 Nuts 117 Tops	148	58	35	28	131 Grain 727 *Karbi*	48	99	124 Grain 939 *Karbi*
A—5 . .	32	27	191 Grain 1,268 *Karbi*	222 Nuts 114 Tops	106	74	29	31	130 Grain 878 *Karbi*	31	79	124 Grain 903 *Karbi*
Average per acre outturn in lbs. .	438	323	1,680 Grain 11,122 *Karbi*	1,950 Nuts 1,142 Tops	1,220	614	336	372	1,440 Grain 8,878 *Karbi*	370	806	1,446 Grain 1,016 *Karbi*

Treatments—

1. Cotton after groundnut, alternating from year to year
2. Cotton, then *juar*, then groundnut
3. Cotton for two years running, then *juar* and then groundnut
4. Cotton for two years running and then *juar*
5. Cotton one year followed by *juar* the next
6. Cotton followed by cotton year after year
7. Cotton for two years running and then groundnut
8. Cotton followed by cotton year after year
   This is the same as No. 6 started in 1925-26 to corroborate the results of No. 6

NOTE.—Blanks in yield columns under 1926-27 are due to failure owing to excessive water logging

TABLE VI—*contd.*

Yield of each of the five replicates of rotational experiments from 1923-24 to 1930-31—contd.

Replication	Treatment No. 5 Outturn per $\frac{1}{10}$ acre plot in lbs.							Treatment No. 6 Outturn per $\frac{1}{10}$ acre plot in lbs.							
	Cotton 1924-25	*Juar* 1925-26	Cotton 1926-27	*Juar* 1927-28	Cotton 1928-29	*Juar* 1929-30	Cotton 1930-31	Cotton 1923-24	Cotton 1924-25	Cotton 1925-26	Cotton 1926-27	Cotton 1927-28	Cotton 1928-29	Cotton 1929-30	Cotton 1930-31
A—1	45	187 Grain 673 *Karbi*	60	144 Grain 727 *Karbi*	44	164 Grain 818 *Karbi*	36	19	33	17	29	47	25	38	37
A—2	34	172 Grain 502 *Karbi*	35	116 Grain 731 *Karbi*	45	156 Grain 808 *Karbi*	31	26	22	25	26	52	27	42	46
A—3	37	169 Grain 565 *Karbi*	34	131 Grain 535 *Karbi*	41	130 Grain 688 *Karbi*	31	16	22	11	25	53	18	32	38
A—4	29	265 Grain 492 *Karbi*	28	115 Grain 463 *Karbi*	42	140 Grain 794 *Karbi*	28	36	33	29	21	54	27	60	39
A—5	42	162 Grain 492 *Karbi*	38	118 Grain 666 *Karbi*	38	183 Grain 862 *Karbi*	33	22	17	13	13	71	29	44	49
Average per acre outturn in lbs.	374	1,910 Grain 5,448 *Karbi*	390	1,258 Grain 6,244 *Karbi*	420	1,546 Grain 7,940 *Karbi*	318	238	254	190	228	554	252	432	418

Treatments—

1. Cotton after groundnut, alternating from year to year
2. Cotton, then *juar*, then groundnut
3. Cotton for two years running, then *juar* and then groundnut
4. Cotton for two years running and then *juar*
5. Cotton one year followed by *juar* the next

Treatments—

6. Cotton followed by cotton year after year
7. Cotton for two years running and then groundnut
8. Cotton followed by cotton year after year
   This is the same as No. 6 started in 1925-26 to corroborate the results of No. 6

NOTE.—Blanks in yield columns under 1926-27 are due to failure owing to excessive water logging

TABLE VI—*concld.*

Yields of each of the five replicates of rotational experiments from 1923-24 to 1930-31—concld.

Replication	Treatment No. 7 Outturn per 1/10 acre plot in lbs.						Treatment No. 8 Outturn per 1/10 acre plot in lbs.					
	Cotton 1925-26	Cotton 1926-27	Groundnut 1927-28	Cotton 1928-29	Cotton 1929-30	Groundnut 1930-31	Cotton 1925-26	Cotton 1926-27	Cotton 1927-28	Cotton 1928-29	Cotton 1929-30	Cotton 1930-31
A—1	28	42	217 Nuts 138 Tops	85	28	165 Nuts 263 Tops	33	54	72	40	40	47
A—2	34	61	244 Nuts 147 Tops	89	43	188 Nuts 363 Tops	40	60	80	37	59	50
A—3	43	37	262 Nuts 147 Tops	93	70	177 Nuts 307 Tops	40	40	85	35	45	48
A—4	52	44	238 Nuts 146 Tops	91	44	168 Nuts 235 Tops	48	41	76	36	39	41
A—5	48	27	209 Nuts 138 Tops	93	80	155 Nuts 273 Tops	29	38	68	33	38	47
Average per acre outturn in lbs.	410	422	2,340 Nuts 1,452 Tops	902	430	1,706 Nuts 2,882 Tops	380	466	762	362	442	466

Treatments—

1. Cotton after groundnut, alternating from year to year
2. Cotton; then *juar*, then Groundnut
3. Cotton for two years running, then *juar* and then groundnut
4. Cotton for two years running and then *juar*
5. Cotton one year followed by *juar* the next
6. Cotton followed by cotton year after year
7. Cotton for two years running and then groundnut
8. Cotton followed by cotton year after year
   This is the same as No. 6, started in 1925-26 to corroborate the results of No. 6

NOTE.—Blanks in yield columns under 1926-27 are due to failure owing to excessive water logging

A NOTE ON A GROWTH ABNORMALITY OF PUNJAB-AMERICAN COTTONS.

BY

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(Received for publication on 9th February 1933.)

During certain years American cotton grown in the Canal Colonies of the Punjab are affected by a peculiar growth abnormality which appears to be of the nature of a virus disease. The attacked plants show very characteristic symptoms. The internodes, petioles and leaves are extremely reduced in size. The leaves are crinkled, deformed and discoloured and yellowish patches give the leaves a characteristic mosaic appearance. The reduction in the size of leaves is also accompanied by a reduction in the number of lobes. The stipules are usually of the normal size, but are very light in colour. The floral organs are very much reduced in size. The apicalyx is discoloured, but not deformed. The shedding of buds and bolls is very high leading to a high degree of sterility of the plant, and the few bolls which are formed are very small in size and give but few viable seeds. The attack may either be confined to a few limbs, or the entire plant may be affected. The degree of severity of attack on the shoot is marked by a fairly proportionate reduction in the size of the root system.

It has been ascertained that the disease is similar to 'stenosis' or 'smalling' described by Cook [1924] in the U. S. A. The attack is confined to American varieties while the indigenous cottons seem absolutely immune.

The attack was first noticed during 1930, but it practically disappeared during 1931, and appeared again with startling severity during 1932. The extent of damage done can be judged from the fact that in several fields at Lyallpur in which counts of the abnormal plants were made nearly fifty per cent. of the plants were affected.

The attack commences when the plants are six weeks old, and the disease is very virulent during August.

REFERENCE.

Cook, O. F. (1924) *J. Agric. Res.* **28** No. 8.

A NOTE ON THE CHROMOSOME NUMBERS IN CLUSTER BEANS, *CYAMOPSIS PSORALIOIDES* D. C.

BY

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Introduction.

As part of a campaign for the improvement of vegetables in Madras, the Millet Section undertook the study of the cluster beans and that of certain other leguminous vegetables, with a view to their improvement. The need was keenly felt for a knowledge of the chromosome numbers of *Cyamopsis psoralioides*, and as no determination of the chromosome number of this pulse had been published, the present study was undertaken.

Materials and methods.

Materials were collected from five distinct varieties representing differences in plant habit, pubescence, and pod size. Young flower buds collected at intervals of one hour from 7 a.m. to 12 noon were killed in Bouin's fluid as modified by Allan. A few drops of saturated solution of lithium carbonate in water were added during each dehydration stage. Paraffin sections were cut at 10μ thickness and stained in Heidenhain's iron-alum hæmatoxylin; the sections were left for two hours in a saturated solution of lithium carbonate prior to mordanting.

Observations.

Flower buds about 2 mm. in width and 3 mm. in length were found to give good division stages. The first divisions were seen in buds collected between 7 a.m. and 9 a.m. and second divisions between 9 a.m. and 11 a.m. The spireme is rather thin and darkly stained. At synapsis the chromatin threads take less stain than the nucleolus. The chromosomes at diakinesis are very darkly stained.

Several good cell plates were met with. In each variety chromosome counts were taken on an average of 50 pollen mother cells in first and second divisions.

Seven was found to be the haploid number in all varieties. Chromosomes counted in diakinesis also gave seven pairs in each nucleus. A few of the cells of the ovary wall in division were observed and the chromosomes counted. In each of these cells 14 chromosomes could be counted easily.

The cluster bean (Cyamopsis) is usually classified under Galegeæ of Papilionatæ [Gamble, 1918], a tribe which is poor in edible legumes. The closely related tribe Vicieæ, though it includes such pulses of economic importance as Vicia, Lens, Lathyrus, Pisum, and Cicer, and which is also characterised by a basal chromosome number 7 [Gaiser, 1930] does not include any characteristic legume, the tender immature pods of which are consumed whole. On the other hand, the well-known vegetables Dolichos and Phaseolus with characteristic edible tender pods are grouped under the Phaseoleæ with basal chromosome numbers 11 and 12 [Rau, 1929 ; Gaiser, 1930]. The improvement of the poor man's vegetable, the cluster bean, is exceedingly desirable because of its hardy qualities but its improvement by hybridization is rendered difficult by the fact that the Vicieæ group contains no species with the desired characters whilst hybridization with species of the Phaseoleæ group is difficult or impossible owing to difference in chromosome numbers.

Summary.

1. The chromosomes of the cluster bean, (*Cyamopsis psoralioides*) are best seen in buds fixed between 7 a.m. and 9 a.m.

2. The haploid number as counted in pollen mother cells is seven.

3. The diploid number, as counted in cells of the ovary wall, is 14.

4. These numbers are similar to those of the genera, in the tribe, Vicieæ of Papilionatæ.

References.

Gaiser, L. O. (1930) . . . . *Bibliographia Genetica* **6**, 248-51.

Gamble, J. S. (1918) . . . . Flora of the Presidency of Madras, Part II, pp. 275—77, London.

Rau, N. S. (1929) . . . . *J. Ind. Bot. Soc.* **8**, 201.

ABSTRACTS

Observations on *Tolyposporium penicillariæ* Bref. (The bajri smut fungus). S. L. AJREKAR, and V. N. LIKHITE. (*Current Sci.* **1,** 215, 1933).

The bajri smut fungus has been grown on different (named) culture media. Spore-balls germinate without any resting period at least on artificial media.

Infection takes place at the time of flowering; but unlike the loose smut of wheat, no dormant mycelium is found within the grain, since sori develop in about two weeks from the date of inoculation. In the affected grain the fungus mycelium is found between the pericarp and the aleurone layer, and eventually gives rise to spore-balls formed from its contents. The source of primary infection in the field has not yet been ascertained.

Seed treatment has failed to check the smut. (B. N. U.)

A study of the Mucorineae of the City of Bombay. S. L. AJREKAR, and K. DHARMARAJULU. (*J. Ind. Bot. Soc.* **10,** 29-34. 1931).

The writers describe briefly seven species of the Mucorales isolated from dungs of various (named) animals in the city of Bombay. One of these is a new species and has been described by Lender under the binomial, *Mucor indicus*.

The two strains of the heterothallic species, *Mucor racemosus* Fr., could be distinguished by a slight difference in the luxuriance of their growth; but it is considered doubtful whether "there is any constant dimorphism in the heterothallic Mucors in respect to morphological characters". (B. N. U.)

Control of onion and chillie thrips in the light of recent researches. T. N. JHAVERI, and A. K. B. CAZI. (*Poona Agric. Coll. Mag.* **23,** 57-58. 1931).

Brief notes are given on the life-history, habits and control of onion thrips, *Thrips tabaci*, and chillie thrips, *Scirtothrips dorsalis*.

The life-history of the two insects is similar, and occupies from 3 weeks to a month depending upon temperature and available nitrogen and water content of the soil. They reproduce by parthogenesis. Eggs are laid in the tissue of the leaf just below the epidermis, and can be seen when the leaf is treated with xylene. Both the thrips are negatively heliotropic, and prefer to feed and shelter in shady places.

The chillie thrips are negatively geotropic, especially the second instar nymph and the adult. This explains why the population of chillie thrips tends to be the densest at the top of the plant.

Onion thrips have a wide range of alternative food plants including the Cruciferæ. No alternative food plants of the chillie thrips have yet been discovered. None of the local varieties of chillies was immune from the attack of thrips. Soil and weeds act as carry-over of these pests.

Control measures of these pests based on cultural practices are indicated. (B. N. U.)

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ORIGINAL ARTICLES

RUSTS OF WHEAT AND BARLEY IN INDIA.*

A study of their annual recurrence, life-histories and physiologic forms.

BY

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(Received for publication on 29th August 1933)

(With Plate LXXV and one Map)

This article has been written at the instance of the Imperial Council of Agricultural Research as a summarized account of the progress of investigations, on different aspects of the problem since April 1930.

Wheat in India suffers from all the three rusts (*Puccinia glumarum* Erikss., and Henn., *P. triticinia* Erikss., and *P. graminis* Pers.) known on that host. The yellow rust (*P. glumarum* Erikss., and Henn.) and the black (*P. graminis* Pers.) are also common on barley but the dwarf rust of barley (*P. simplex*) is very rare. Black rust of oats has only recently been found in the Nilgris during the course of these investigations.

Study of the annual recurrence of rusts on wheat and barley in the plains of India was started by the writer in the year 1923 and the relative importance of the various factors concerned has been discussed in previous publications [Mehta, 1929, 1931].

In the first publication mentioned above, the writer has already reviewed earlier and contemporary work done on this problem and it is unnecessary therefore to enter into those details again.

During the period under report, work on the rusts of wheat and barley was extended in the following directions :—

(*i*) For the study of incidence of rusts in relation to their dissemination, a large number of stations in different parts of the country were visited,

* The previous article on the "Annual outbreaks of rusts of wheat and barley in the plains of India" was published in this *Journal*—Vol. I, Part III, 1931.

in addition to regular observations that were made in the Kumaon and Simla hills.

(*ii*) Dissemination of rusts has also been studied during the last three years with the help of slides that were exposed in aeroscopes (Plate LXXV, figs. 1-3) at different stations. During 1932-33 aeroscope slides were exposed at 46 stations over the area of wheat cultivation in the country as a whole. During the last two winters cellophane strips smeared with a little vaseline were sent up on ordinary kites frequently at Agra in order to catch rust spores from the air. This device (Plate LXXV, fig. 4) proved more satisfactory than the automatic spore trap* used in earlier trials. With the spore trap a slide could not be exposed for more than five minutes, whereas the kite can be kept in the air for 2-3 hours at a stretch.

(*iii*) A preliminary study of the physiologic forms of brown and black rusts of wheat was started in 1930-31, in order to see if samples of rusts from different parts of the country showed indications of the occurrence of a large number of forms, as has been found to be the case in America.

Tests made with such samples showed striking resemblances in their parasitic behaviour on the differential hosts used by the American workers [Stakman and Levine, 1922; Mains and Jackson, 1926]. During 1932-33 a detailed study was made of 35 samples of black rust and 15 of brown.

Nineteen samples of yellow rust were also tested on a set of Indian, Canadian and German varieties of wheat in order to select differential hosts suitable for further work on this rust.

I. Incidence of rusts in relation to their annual outbreaks.

As stated above, this aspect of the problem has been under study for nearly ten years now and a considerable amount of information has been obtained on over-summering of rusts in the uredo-stage in hills in different parts of the country.

During the last three years incidence of rusts has been studied regularly in the Kumaon and Simla hills, with the object of following up their outbreak on crops in those areas. In addition to field observations, miniature plots were started at three different altitudes in order to study the influence of weather conditions on the viability of uredospores at each locality throughout the year.

Two of the miniature plots were attached to the laboratories, *i.e.*, one at Almora (5,400 ft. above sea level) and the other at Simla (7,000 ft.) and the third was established at Muktesar (7,600 ft.). In September 1932 another miniature plot was started at Narkunda (9,200 ft.). This study has given valuable information on

* The mechanism of the spore trap is explained in a note by Mr. G. Chatterji, Meteorologist in charge of the Upper Air Observatory, Agra (*Ind. J. Agric. Sci.* Vol. I, Part III, 1931.)

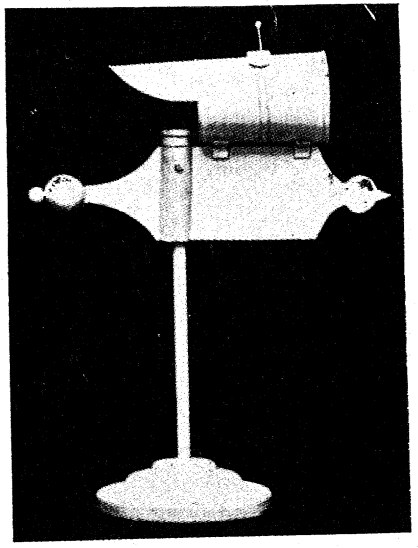

Fig. 1.

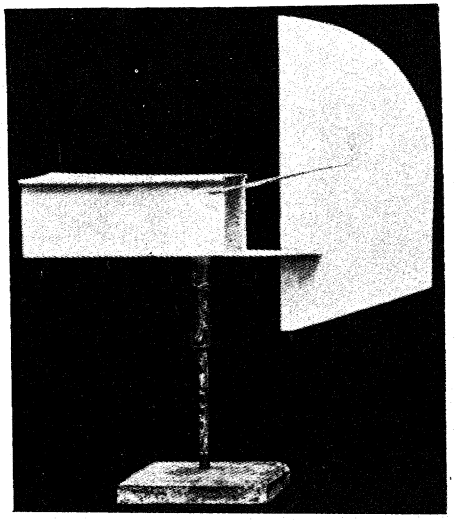

Fig. 2.

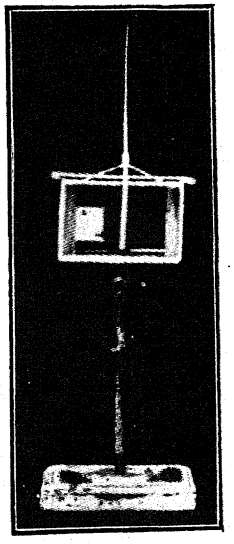

Fig. 3.

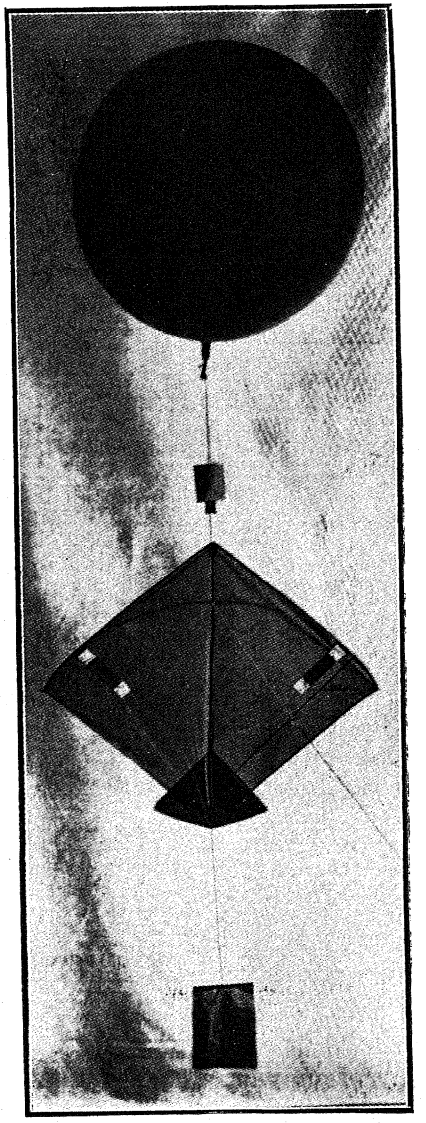

Fig. 4.

PLATE LXXV.

oversummering and overwintering of all the rusts under study, besides throwing light on the more intricate phenomena connected with their outbreaks on the plains.

The present state of our knowledge of the annual recurrence of rusts in this country may be summarized as follows:—

(1) YELLOW RUST (*P. glumarum* ERIKSS., AND HENN.).

The writer has already recorded in previous publications that this rust cannot stand warm weather. It has not been found to oversummer below 6,000 ft. At Almora (5,400 ft.), as was expected, this rust died every year in May-June in the miniature plot as well as in cultures under protection. At Simla (7,000 ft.) it showed poor infection and rather low germination during summer in miniature plots but was not killed. At Muktesar (7,600 ft.) also it showed poor germination in June but recovered soon after that. During winter it has been found to flourish at all the three places mentioned above. In fact this rust can stand cold better than the other two as has been recorded before.

It is interesting to note that during the last three years this rust has been found to break out on the new crop as early as November-December in the neighbourhood of Simla. Normally it breaks out on the new crop by the middle of January even at lower altitudes in the Simla hills. In the Kumaon hills also first outbreaks of this rust have been found to occur in November-December at Muktesar and it is fairly common at lower altitudes 1-2 months later.

The writer has recorded before that at the foot of the hills this rust appears on the new crop during January-February each year and on the plains a few days later.

At comparatively high altitudes there is plenty of this rust on volunteer wheat and barley during summer and at the time of new sowings (October-November). The infection of the new crop at those altitudes as early as November-December therefore does not need any explanation. After infection of the new crop it seems to spread during early winter in spite of a comparatively long incubation period at that time of the year and is then blown down to the plains where it breaks out on the crops during January-February.

(2) BROWN RUST (*P. triticina* ERIKSS.).

This rust can stand warm weather better than yellow and has been found to survive during the last three summers at Almora (5,400 ft.).

Both at Simla and Muktesar it flourishes throughout summer.

During winter this rust seems to be unaffected at Almora and even at Simla, whereas at Muktesar its viability goes down considerably although it is not killed by the cold at that altitude either.

As regards its outbreak on the new crop (sown in October-November) it is interesting to note that at Muktesar (7,600 ft.) it has never been noticed before April. At lower altitudes in the Kumaon hills like Almora it breaks out on the new crop by the month of March. At higher altitudes in the Simla hills like Mattiana (7,900 ft.) and Narkunda this rust has been found only in traces as late as the last week of May. In the neighbourhood of Simla and at lower altitudes it normally breaks out in the month of March. In the year 1931 it was noticed on the new crop near Simla as early as the end of November but even up to the end of March 1932 it showed hardly one per cent. crop infection.

It is important to note in this connection that in some of the submontane districts of United Provinces this rust breaks out as early as December-January.

In order to make sure, if brown and black rusts that were found to survive during summer at Almora (5,400 ft.), can oversummer at lower altitudes also, an intensive search was made for them during May and June this year in the Kumaon hills. The information obtained is very valuable and clearly indicates that both these rusts can oversummer at altitudes as low as 3,500-4,000 ft. under natural irrigation. In the case of black rust, which can stand warm weather even better than brown, a fairly heavy infection was found on volunteer wheat at Majhera (3,200 ft.) on the banks of the Kosi in the third week of June, the hottest month of the year and nearly two months after harvest. Brown rust has been found to survive during May-June this year at ten different places in the Kumaon hills at altitudes of 3,500-4,000 ft. All these places are situated on the banks of a river or its tributary.

(3) Black rust (*P. graminis* Pers.).

This rust can stand warm weather even better than brown and has been found to flourish each year during summer at Muktesar, Simla and even Almora (5,400 ft.).

During winter it suffers more than the other two and at Muktesar its viability fell down as low as one per cent. in December-January in the year 1931-32. In the year 1932-33 it was killed by the winter cold in the miniature plot at Muktesar and there was no trace of it on the crops either at that locality up to the end of March 1933.

At Narkunda (9,200 ft.) there was no trace of this rust in miniature plot (started in September 1932) or on the crops even up to the third week of May 1933. It is almost certain that at that altitude this rust cannot overwinter.

At Simla it has been found to overwinter during the last three years although its viability during December-February fell down to 2-5 per cent.

At Almora it flourished during each winter in spite of a lower viability than that of the other two rusts.

As regards its outbreak on the new crops in hills it may be mentioned that at Almora it appeared in the 2nd-3rd week of March during the last three years. At Simla it was noticed on March 29th this year near rusted volunteer wheat on which it had been found to overwinter. In the year 1932 it was absent up to the end of March and appeared in April. At Muktesar it has not been found before April any year and at Mattiana (7,900 ft.) and Narkunda (9,200 ft.), in the Simla hills, it appears later still, *i.e.*, May-June.

As recorded above, this rust has recently been found to survive during May-June on volunteers at an altitude as low as 3,200 ft. on the banks of a river in the Kumaon hills. In that area it has been found at as many as 16 places at altitudes of 3,000-4,000 ft. on volunteers during the hottest part of the year.

It is important to note that like the brown rust this also breaks out in the submontane districts of the United Provinces, Bihar and certain parts of Bombay-Deccan during December-January and sometimes a little earlier in the latter area.

(4) Brown and black rusts—general observations.

At the outset it may be mentioned that the phenomena connected with the annual outbreaks of these two rusts on the plains are not so clear as in the case of yellow rust. Both these rusts oversummer in the uredo-stage at such altitudes which the yellow can stand. As a matter of fact they can oversummer even at altitudes 2,000-3,000 ft. lower than those for yellow as they can stand warm weather better than the latter.

Judging from the late appearance of these rusts at comparatively high altitudes in the hills, wherefrom yellow rust in all probability disseminates to the plains, one cannot attribute their outbreak on the plains to the inoculum oversummering and then struggling against cold during winter at those altitudes.

The writer has recorded before that these two rusts are disseminated to the plains in all probability, from comparatively low altitudes, where on account of a milder winter their uredospores, occurring at the time of sowing, infect the new crops rather early in the season (November-December). After their outbreak on the crops both these rusts should be able to spread from plant to plant during early winter at such altitudes.

Otherwise it is difficult to explain their outbreak as early as December-January year after year in some of the submontane districts of the United Provinces and Bihar.

As stated above black rust is also known to break out in certain parts of Bombay-Deccan during the same period and sometimes even earlier, although there are no hills with high altitudes in that area.

The writer is fully convinced of the possibility of oversummering of these two rusts, as mentioned above at altitudes in the neighbourhood of 3,000-4,000 ft. above sea level, certainly at such localities, which are adequately protected against direct sun by a favourable aspect and have plenty of natural irrigation or a fair amount of rainfall, well distributed during the critical period. Under moist conditions oversummering of black rust, at any rate seems likely at such localities, where the maximum summer temperature may only casually rise to 90-95°F. as indicated by some recent observations in the Kumaon hills.

Judging from the fact that these two rusts normally do not appear before March at the comparatively high altitudes and not before May-June at places like Mattiana and Narkunda, it is clear that *Thalictrum* and *Berberis*, the two alternate hosts, play little part as far as their outbreak on the plains is concerned.

As a matter of fact excepting the Punjab, crops on the plains are normally harvested by the end of March and, as stated above, these rusts appear in certain parts of the country as early as December-January. By the time they break out at comparatively high altitudes in the hills, where the suspected species of *Berberis* and *Thalictrum* occur, normally these rusts are in an epidemic stage on the plains as a whole.

On the strength of the study of incidence of rusts made in other parts of the country during the last three years it may safely be stated that—

(i) In Siwalik range as well as Murree hills there are foci of infection, where the rusts under study oversummer as well as survive through winter.

(ii) So far no evidence of oversummering has been obtained in the Central Provinces and it seems that the greater part of the inoculum for that area comes from the tract lying on the North.

(iii) In Bombay-Deccan the study of the incidence of black rust made so far is very suggestive of the existence of foci of infection in the Western Ghats.

(iv) All the rusts under study were found by the writer in the Nilgris in December 1931 and they were again observed on the crop as well as volunteers and tillers in October 1932. In the Palni hills also brown and black rusts were found on the crop at that time. It is probable therefore that the wheat and barley crops on the plains of Madras get infected by the inoculum, which disseminates from the Nilgris and the Palai hills.

In the light of the information that has been collected with regard to these two rusts, the writer hopes that further work would soon lead to the location of places of earliest outbreak in each of the important areas, wherefrom they may be disseminating to different parts of the country.

II. Study of the life-histories of brown and black rusts.

(1) Brown rust.

During the last three years several inoculations were made on wheat with accidiospores from *Thalictrum javanicum* found near Simla but the results were always negative. In this connection it is important to note that the species of *Thalictrum*, on which Jackson and Mains [1921] succeeded in producing aecidia by artificial inoculations in America, are not known to occur in this country.

Barclay [1887] found aecidia on *Thalictrum minus* also on the Tibet road. This host was only weakly infected in the experiments conducted by Mains and Jackson.

According to Butler and Bisby [1931] and Barclay [1887] the aecidial material on both *Thalictrum javanicum* and *Thalictrum minus* probably belongs to *Aecidium urceolatum* Cke.

It is interesting to note that the teleuto-stage of this rust is very scarce in this country and often impossible to obtain. So far all germination tests with its teleutospores have been unsuccessful. It is not certain if there is an alternate host of this rust in India.

This rust lives from season to season in the uredo-stage and has been found to oversummer as well as overwinter at Almora, Simla and Muktesar, as stated in the earlier part of this article. The aecidial stage, therefore, does not seem to be essential to the propagation of this rust as well. The writer [1923] recorded a similar observation with regard to the annual recurrence of this rust in England. Arthur [1929] has also expressed that the full rôle of the aecidial stage of this rust is unknown as far as America is concerned and that it is not essential to its propagation, as the species is able to overwinter in the uredo-stage.

(2) Black rust.

During the period under report a large number of inoculations were made on wheat and barley with aecidiospores from the shorter aecidia occurring on *Berberis lycium** and *Berberis aristata** in the Simla hills. Several inoculations were made with aecidial material from *Berberis vulgaris* also, occurring near Narkunda. Most of the inoculations were made at the spot on seedlings, which were carried in rust-proof cases. The material used showed good germination in most of the experi-

* On these three species *Aecidium montanum* Butl. is more common than the shorter aecidia.

ments, which were conducted on different dates. No infection of wheat or barley was obtained. The same applies to inoculations made with aecidiospores from *Berberis coriaria.**

After a large number of trials during 1930-32, germination of the teleutospores of black rust of wheat was obtained at Simla in April last. The material was collected from a miniature plot, where it appeared in November-December 1932. It germinated without artificial freezing, having been exposed to snow and natural conditions of weather during December-March.

As many as 65 leaves of *Berberis vulgaris*, on plants raised from foreign seed and 75 leaves on plants of the same species, transplanted from Narkunda were inoculated with the above material showing 30-50 per cent. germination. Out of the former 14 got infected, whereas no infection took place on the latter.

Inoculations on *Berberis coriaria*, *B. lycium* and *B. aristata* have also yielded negative results so far, possibly due to comparatively warm weather during May-June, when most of the tests were made.

This work is being continued and before long one should obtain infection of such species of *Berberis* as may be connected with the life-cycle of black rust in this country.

Aecidiospores, from aecidia thus produced on *B. vulgaris* raised from foreign seed, were put on wheat and barley and as was expected, rust appeared on both of them. That proves the academic part of the connection, as in Europe and America, between black rust in this country and *Berberis vulgaris*, at any rate, the foreign plant (raised from seed collected by the writer at Cambridge in 1930).

Butler and Bisby [1931] have recorded the aecidial stage (*Aecidium berberidis* Pers.) of black rust on *Berberis vulgaris*†, *B. aristata*, *B. lycium* and *B. umbellata* in the Himalayas.

So far there is no evidence to show that the aecidial material on any of the indigenous species of *Berberis* is connected with black rusts of wheat or barley in this country.

In order to solve the *Berberis* tangle finally, the writer proposes to continue inoculations with germinating teleutospores of this rust on all the four species, mentioned above and some others, if found necessary.

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* On these three species *Aecidium montanum* Butl. is more common than the shorter aecidia.

† Plants of *Berberis vulgaris*, collected at Narkunda, were sent to the Forest Institute at Dehra Dun in order to make sure, which variety it is that occurs at that locality. The Forest Botanist has kindly supplied the following information :—(1) That true *B. vulgaris* Linn. does not occur in India according to Schneider's Revision of the genus *Berberis* in Bulletin de l'Herbier Boissier, Vol. V (1905). (2) According to Parker's Forest Flora of the Punjab with Hazara and Delhi (1924) the Narkunda plants are *B. petiolaris* Wall. (syn. *B. pachyacantha* Koehne). (3) According to Hooker's Flora of British India they are *B. vulgaris*, var. *Ivulgaris* proper.

III. Cross inoculations with yellow rusts of wheat and barley.

As elsewhere, the yellow rust of wheat has failed to infect barley and *vice versâ*.

IV. Cross inoculations with black rusts of wheat, barley, *Bromus patulus* and oats.

(i) Black rust of wheat, as elsewhere, has been found to infect wheat and barley but not rye or oats.

(ii) Black rust of barley infected barley, wheat as well as rye, though only moderately.

(iii) *Bromus patulus*, a wild grass was found infected with black rust in a wheat field at Mattiana in May 1932 for the first time. This rust was cultivated on *Bromus patulus* and tested on the same host, wheat, barley and rye. All the four got infected although the infection on rye was rather mild.

(iv) Black rust was also found on oats in the Nilgris in October 1932. As far as the writer is aware this rust has not been recorded from India before. After cultivation on oats for three generations, it was put on wheat and barley and as expected neither of them got infected, whereas oats were.

Further work is in progress in order to make sure, if *P. graminis secalis* is also present in the black rusts of barley and *Bromus patulus*. Samples of rust from both these hosts have been tested on the differential hosts used by Stakman and his co-workers for physiologic forms of *P. graminis tritici*. The rust on *Bromus* has yielded Forms XV and XLII and that on barley Form LXXV.

V. Dissemination of rusts in relation to first outbreaks on the plains.

The writer [1931] has already recorded results of a preliminary study of dissemination with slides exposed in aeroscopes and those sent up on hydrogen balloons in the year 1929-30. Since then this work has been very much extended and during 1932-33 slides were exposed in 47 aeroscopes at as many as 46 stations (shown in the map) over the area of wheat cultivation in the country as a whole.

Data obtained during the last three years clearly show that at most of the stations uredospores of a particular rust were caught from the air well before its appearance on the crop at those localities.

It is interesting to note that at some of the stations (Charts I-III, pp. 956-962) a fairly large number of spores (20-172) was caught on a slide long before the appearance of rust at those localities.

The following facts are of special interest (Chart III) :—

(1) At Gorakhpur, which is at the foot of the Nepal range, spores of black rust were caught as early as the middle of November and that rust was observed on the crop on January 10th following.

(2) At Patna, which is farther south, the same rust appeared on March 6th and its spores were first caught on January 18th-21st.

(3) At Hoshiarpur, which is at the foot of the Siwalik Range, spores of black rust were caught nearly 2 months earlier than at Lyallpur (central Punjab) and the rust appeared one month earlier at the former station. At Gurdaspur, which is also at the foot of the hills, spores of yellow rust were caught nearly one month earlier than at Lyallpur and rust appeared at the latter station nearly 3 weeks later.

Stations in the Punjab show late spore showers and consequently very late appearance of rusts.

The writer hopes that he would be supplied with wind trajectories by the Meteorological Department and with the help of that information it may be possible to follow up the directions of dissemination over each of the large areas in the country from year to year. Such a study extended over a few years should be a considerable help in understanding the epidemiology of rusts and also the sequence of their appearance in the different parts, as well as the absence of one rust or the other, in certain parts of the country from year to year.

Physiologic forms of rusts of wheat and barley.

(*a*) Black rust.

So far 35 samples of this rust have been studied in detail, *i.e.*, 33 samples of rust of wheat, one each of barley and *Bromus patulus*. Four samples out of these have not yet been fully analysed and are under study at present. It would be clear from Table I, appended herewith, that the collection is fairly representative.

So far only four physiologic forms of *P. graminis tritici* have been met with, *i.e.*, Form XV, XL, XLII and LXXV, as described in Stakman's key. Their distribution is as follows :—

All the four forms occur in the Punjab, United Provinces and Bombay-Deccan. Form XV is found also in Bihar and Madras ; Form XL in Hyderabad-Deccan and Madras ; Form XLII in Central Provinces, Rajputana, Hyderabad-Deccan and Madras.

In addition to the stock samples, referred to above, 21 single spore cultures started from different samples or their isolations have been tested and it is interesting to note that the same four forms have been picked up from those cultures.

Another interesting feature of the study is that seven out of the twelve differential hosts used by the American workers [Stakman, Levine and Leach, 1919 ; Stakman and Levine, 1922], have been found to be susceptible to each of the 35 samples, their isolations as well as all the single spore cultures.

(*b*) Brown rust.

Fifteen stock samples from different stations and ten single spore cultures of this rust have been studied in detail. Only two physiologic forms have been met with so far, *i.e.*, Form X and a New Form, which is not described in the latest key of Mains, Jackson and Johnston of U. S. A. [1926]. Two samples have not yet been fully analysed and are under study at present.

Both the forms occur in the Punjab and United Provinces and the New Form has also been found in Bihar and the Central Provinces.

Form X has been picked up only in one single spore culture, the other nine cultures gave the New Form (Table II).

(*c*) Yellow rust.

Nineteen samples of this rust and one single spore culture have been tested on a set of Canadian, German and Indian varieties with a view to select suitable hosts for the study of physiologic forms of this rust.

The types of infection produced by different samples on the five German varieties, show indications of two out of the four forms described by Allison and Isenbeck [1930] occurring in this country. As far as the Canadian and Indian varieties are concerned, there are clear differences in the types of infection produced by different samples on some of the hosts.

For further work on this rust, differential hosts that have recently been selected by Gassner and Straib [1932] in Germany will be used in order to identify the different physiologic forms that may be occurring in this country.

Judging from the fact that in this country the rusts under study are propagated from season to season largely by their uredospores, the writer feels convinced that the number of physiologic forms of each rust should not be large. It has been demonstrated in America that new physiologic forms originate by hybridization on the alternate host, *i.e.*, on *Berberis* in the case of black rust.

In India the total absence of any species of *Berberis* and *Thalictrum* on the plains, which cover nearly 95 per cent. of the entire area under wheat, is another strong evidence in favour of the above contention. In the case of yellow rust no alternate host has so far been discovered anywhere.

The circumstantial evidence connected with the outbreak of brown and black rusts on the plains indicates that the alternate hosts of these rusts play little part in their annual recurrence.

Further, the fact that seven out of the twelve differential hosts for black rust have been found to be susceptible to every sample or its isolation tested so far, is another indication of the occurrence of a small number of forms of that rust.

A detailed account of work on physiologic forms of the rusts of wheat and barley, along with tables showing types of infection, will be published at a later date, when a sufficiently large number of samples of each rust has been tested. Arrangements have been made for this study to be carried out throughout the year between the laboratories at Simla and Agra and it would be possible to test 150-200 samples per year in future.

The writer wishes to express his warmest thanks to the following workers for their kind help and co-operation in these investigations :—

(1) Drs. E. C. Stakman and C. O. Johnston of the United States Department of Agriculture.

(2) Drs. C. C. Allison and K. Isenbeck of the University of Halle, Germany.

(3) Dr. M. Newton ot the Rust Research Laboratory, Winnipeg, Canada.

(4) Dr. E. J. Butler of the Imperial Mycological Institute, London.

(5) Prof. F. L. Engledow and Mr. F. T. Brooks of the University of Cambridge.

(6) Directors of Agriculture in the Punjab, United Provinces, Bihar and Orissa, Central Provinces, Bengal, Assam, Bombay-Deccan, Hyderabad-Deccan, Madras, Mysore and Baroda.

(7) Agricultural Officer, North-West Frontier Province ; Agricultural Officer, Baluchistan ; Agricultural Officer, Sind ; The Imperial Mycologist, Pusa ; Director-General of Observatories ; Agricultural Meteorologist and the Meteorologist in charge of the Agra Observatory.

(8) All other officers of the Department of Agriculture and non-officials, who have helped in the exposure of slides in aeroscopes, and the collection of rust samples.

The writer wishes to record his appreciation of the help rendered by his research assistants in the investigations under report.

For the continuance of this work during 1930-1933 with necessary expansion of its scope, the Imperial Council of Agricultural Research, India, kindly made two grants to the total value of Rs. 51,500. The officers of the Council have been very kind and accommodating and the writer wishes to express his warmest thanks to them for facilitating the work in every way.

Summary.

A brief account of the progress of investigations on rusts of wheat and barley since 1930 has been given above especially for the information of overseas workers, who may be interested in the problem.

(1) Annual outbreaks of yellow rusts of wheat and barley on the plains of India are caused by wind-blown uredospores which are disseminated from comparatively high altitudes in the hills, where they oversummer.

(2) As far as the plains are concerned, species of *Berberis* and *Thalictrum*, the alternate hosts for black rust of cereals and the brown rust of wheat respectively seem to play little part in the yearly origin of those rusts.

(3) The rôle of *Thalictrum* as an alternate host is yet very doubtful.

(4) *Berberis vulgaris*, raised from seed collected in England, has been successfully infected by sporidia of black rust of wheat in this country. Wheat and barley were infected by aecidiospores thus produced. The rôle of indigenous species of *Berberis* in fresh outbreaks of black rust is yet under study.

(5) Brown rust of wheat and the black rusts of wheat and barley are in all probability disseminated to the plains from comparatively low altitudes, where on account of a milder winter their uredospores occurring at the time of sowing cause outbreaks on the new crop rather early in the season (November-December). Both these rusts have recently been found to oversummer at altitudes of 3,500-4,000 ft. under natural irrigation in the Kumaon hills.

(6) Study of rust dissemination with the help of slides exposed in aeroscopes, practically all over the country and those sent up on kites, has yielded valuable information with regard to the directions along which dissemination takes place.

(7) So far only 35 samples of black rust and 15 of brown have been studied in detail. These samples have yielded only four forms of black rust and two of brown. Single spore cultures from samples of these two rusts have given no additional forms.

(8) There is a strong evidence, both circumstantial and scientific, in support of the writer's contention, that the number of physiologic forms of the rusts under study should not be large in this country.

(9) The complete absence of the alternate hosts for brown and black rusts on the plains, which occupy nearly 95 per cent. of the area under wheat and barley, is the most hopeful feature of the problem as far as measures of control are concerned.

(10) Propagation of rusts by uredospores from season to season, which takes place in some other countries, is impossible on the plains of India in general, on account of the intense heat of summer. It is almost impossible for the uredospores to survive after the harvest on the plains as a whole.

(11) On the basis of data obtained so far the writer is fully convinced of the fact that the problem under study is unique, as far as this country is concerned and the possibility of controlling rusts by the methods already suggested, is infinitely greater than elsewhere.

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Explanation to Plate LXXV.

Fig. 1 . . . . The half-slide aeroscope designed by G. Chatterji, Meteorologist I-C, Upper Air Observatory, Agra. This instrument was used in 1929-30 and 1930-31 and is still in use at four stations.

Fig. 2 . . . . Aeroscope or a weather-vane type of spore trap originally designed by P. M. Simmonds and used by the Canadian workers. This model has been in use for the exposure of stationary slides at most of the stations since 1931.

Fig. 3 . . . . The above as seen from one end indicating the position of the glass slide, which is smeared with a little vaseline.

Fig. 4 . . . . A kite in action, designed by the writer. Two cellophane strips smeared with a little vaseline are pasted on the kite, which is kept a captive. Photograph shows the flag, which is intended for calculating the height up to which the kite flies. In most of the flights the kite reached an height of nearly 1,500 ft. above the ground.

TABLE I.

Showing the physiologic forms of Puccinia graminis tritici *isolated from 33 samples of Black Rust of wheat, a sample of Black Rust of barley and one sample of Black Rust of* Bromus patulus

A. *Black Rust of wheat*

Serial No.	Province	Locality	Original host	Forms picked up	Remarks
1	Punjab	Narkunda	Local	XV	* One more isolation is yet to be tested.
2	,,	Mattiana	,,	XV and XL	
3	,,	Simla (1930-31)	,,	LXXV and XL.	
4	,,	Simla (1931-32)	,,	XL and XLII.	
5	,,	Rawalpindi	Utac U. S. A.	XL	
6	,,	Lyallpur	Local	XLII	
7	,,	Ditto	Macoroni	XLII	
8	,,	Ditto	Blue chaff	XL and XLII.	
9	,,	Gurdaspur	Punjab Type 14	XL	
10	Sind	Sakrand	C. P. H. 47	XLII	
11	United Provinces.	Muktesar (1931-32)	Local	XL and XLII.	
12	,,	†Muktesar-Almora (1930-31).	,,	XL, XLII and LXXV.	
13	,,	Almora	,,	XV	
14	,,	Cawnpore	,,	XLII and LXXV.	
15	,,	Agra	,,	XL	
16	,,	Agra (late sample)	,,	XL	
17	,,	Allahabad	,,	XV	* One of the isolations is yet to be retested.
18	Bihar	Pusa	Pusa 101	XV	
19	Central Provinces.	Saugor	Local	XLII	
20	,,	Jubbulpore	,,	XLII	
21	,,	Nagpur	,,	XLII	
22	Rajputana	Ajmer	,,	XLII	
23	Bombay-Deccan.	Poona	Pusa 4	XL and XLII	
24	,,	Ditto	Bansi	XLII	
25	,,	Dharwar	Pusa 4	XV and LXXV.	
26	,,	Ditto	Bansi	XL and XLII	
27	,,	Niphad	Do.	XLII	

* One isolation in the case of each of these samples is yet to be tested in order to make sure if there is some other Form besides the one already identified.

† From miniature plot at Almora, inoculated with sample from Muktesar.

TABLE I—*contd.*

A. *Black Rust of Wheat*—contd.

Serial No.	Province	Locality	Original host	Forms picked up	Remarks
28	Bombay-Deccan.	Niphad . . .	Pusa 4 . .	XLII	
29	Hyderabad-Deccan.	Hyderabad . .	140 C. P. . .	XL and XLII	
30	Madras .	Bellary . . .	Local . . .	XL . .	* One of the isolations is yet to be retested.
31	,, .	Kotagiri . . .	Wheat tiller 1931 .	XLII and XV.	
32	,, .	Kodai Kanal (1932) .	Loccal . . .	XLII	
33	,, . .	Ketti (1932) . .	,, . .	XV . .	* One of the isolations is yet to be retested.
		B. *Black Rust of barley and* Bromus patulus			
1	United Provinces.	Almora . . .	Barley . .	LXXV	
2	Punjab .	Mattiana .	*Bromus patulus*. .	XV and XLII.	

* One isolation in the case of each of these samples is yet to be tested in order to make sure if there is some other Form besides the one already identified.

Comparison of means of infection given in Stakman's key and those obtained from single spore cultures tested here so far.

Form	Means of infection.	Little club	Marquis	Reliance	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli	Remarks
XV	From Stakman's key.	4	4—	4=	3+	4=	4=	4=	3++	3++	3++	4±	1=	
	S. S. test .	4—	4	4	4	4	4	4	4	4	4	4	2	
XL	From Stakman's key.	4+	4+	4	4+	4+	4+	4	4=	4	0;	4=	1=	
	S. S. test .	4—	4	4	4+	4+	4+	4	4	4	1—	4	1=	
XLII	From Stakman's key.	4	4	0	0;	4+	4	4	4	4	4=	2=	4 c	
	S. S. test .	4	4	0;	0;	4	4	4	4	4	4	2=	4 c	
LXXV	From Stakman's key.	4	3+	2+	0;	3+	3+	3+	4—	3+	1	0;	1	*
	S. S. test .	4	4	0;	0;	4	4	4	4	4	1	2	1	

* There are differences between the types of infection on Reliance and Vernal in the two cases. Isolations from Vernal and Einkorn have been tested thrices from different samples and an isolation from Khapli has been tried once. In every case the results were identical.

TABLE II.

Showing the physiologic forms of Puccinia triticina *isolated from 15 samples of Brown Rust of wheat.*

Serial No.	Province	Locality	Original host	Remarks
1	Punjab . .	Narkunda . . .	Local	One isolation is yet to be tested.
2	,, . .	Simla (1930-31) . .	,,	
3	,, . .	Lyallpur . . .	,,	
4	United Provinces .	Muktesar-Almora* (1930-31).	,,	
5	,, .	Muktesar . . .	,,	
6	,, .	Gorakhpur . . .	,,	
7	,, .	Lucknow . . .	,,	
8	,, .	Chandausi . . .	,,	
9	,, .	Almora . . .	,,	
10	,, .	Allahabad . . .	,,	One isolation is yet to be tested.
11	,, .	Cawnpore . . .	,,	
12	,, .	Agra	,,	
13	Bihar . . .	Pusa	,,	
14	Central Provinces.	Nagpur . . .	,,	
15	,, .	Jubbulpore . . .	,,	

Nos, 1, 2, 4, 5, 6, 7, 13, 14 and 15 gave the same Form, *i.e.*, a New Form not described in Mains, Jackson and Johnston's key.

Nos. 3, 10, 11 and 12 . . . yielded Form X.

Nos. 8 and 9 . . . . gave two Forms each, *i.e.*, the New Form and Form X.

* From miniature plot at Almora, inoculated with sample from Muktesar.

Comparison of the means of infection given in Mains, Jackson and Johnston's key and those obtained from the various tests made here.

Form	Means of infection	Malakoff	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	Remarks
X	Mains, Jackson and Johnston's key.	4	4	4	4	4	1—2	1—2	1—2	
	Tests made here . .	4	4	4	4	4	1—2	2—3	1—2	Picked up in stock tests, isolations and one single-spore culture.
New	Tests made here. .	0;	1—2	2—3	0—1	1—2	1—2	0;	1—2	Picked up in stock tests, isolations and 9 single-spore cultures.

Only two physiologic forms have been met with so far, *i.e.*, Form X and a Form not mentioned in Mains, Jackson and Johnston's key, and is therefore styled as a 'New Form.'

CHART I.

Showing the dates of earliest spore shower and the dates of rust appearance at different stations (1930-31).

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
			Aeroscope slides.				
1	Punjab	Lyallpur	14th to 17th Jan. 1931.	...	1st week of April 1931.	...	
2	,,	,,	17th to 21st Jan. 1931.	...	...	...	9th February 1931.
3	United Provinces	Agra	21st to 24th Jan. 1931.	...	22nd February 1931.	...	
4	,,	Cawnpore	17th to 21st Jan. 1931.	...	2nd week of February 1931.	...	
5	,,	,,	17th to 21st Jan. 1931.	...	...	2nd week of February 1931.	
6	,,	,,	28th to 31st Jan. 1931.	...	...	...	2nd week of February 1931.
7	,,	Gorakhpur	1st to 4th Dec. 1930.	...	Last week of December 1930.	...	
8	,,	,,	1st to 4th Dec. 1930.	...	—	Last week of December 1930.	
9	,,	,,	26th to 31st Dec. 1930.	...	..	...	Last week of December 1930.
10	Central Provinces	Nagpur	3rd to 7th Jan. 1931.	14th to 18th Feb. 1931. (31).	21st February 1931.	...	
				Balloon slides.			
1	United Provinces	Agra	20th Dec. 1930.	...	22nd February 1931.	...	

CHART II.

Showing the dates of earliest spore shower and the dates of rust appearance at different stations. (1931-32).

Serial No	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
				Aeroscope slides.			
1	Punjab .	Lyallpur .	9th to 13th Jan. 1932.	...	...	...	22nd January 1932.
2	,,	Gurdaspur .	24th to 27th Dec. 1931.	...	...	...	15th January 1932.
3	,,	Rupar (Shiampur.)	30th Dec. 1931 to 2nd Jan. 1932.	...	...	...	15th January 1932.
4	United Provinces	Agra .	27th to 30th Jan. 1932.	...	3rd week of February 1932.	...	
5	,,	,,	20th to 23rd Jan. 1932.	3rd to 6th Feb. 1932 (30).	...	2nd week of February 1932.	
6	,,	,,	30th Jan. to 3rd Feb. 1932.	..	...	...	2nd week of February 1932.
7	,,	Gorakhpur.	18th to 26th Dec. 1931.	—	...	...	1st week of January 1932.
8	Bihar .	Pusa .	13th to 17th Dec. 1931.	..	5th February 1932.	...	
9	,,	,,	24th to 27th Dec. 1931.	...	...	26th December 1931.	
10	,,	,,	20th to 24th Dec. 1931.	...	...	...	16th January 1932.

CHART II—*contd.*

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
			Aeroscope slides—contd.				
11	Rajputana	Ajmer	14th to 18th Nov. 1931.	...	26th February 1932.	...	
12	,,	,,	18th to 21st Nov. 1931.	...	..	26th February 1932.	
13	Central Provinces	Nagpur	14th to 21st Dec. 1931.	...	6th March 1932.*	...	
14	,,	,,	22nd to 25th Jan. 1932.	...	..	6th March 1932 *	
15	,,	Jubbulpore.	19th to 23rd Jan. 1932.	...	3rd March 1932.*	...	
16	Bombay-Deccan.	Dharwar	22nd to 26th Oct. 1931.	22nd to 26th Oct. 1931 (30).	26th November 1931†.	...	
17	,,	Poona	15th to 19th Oct. 1931.	22nd to 26th Oct. 1931 (41).	2nd week of† November 1931.	...	
18	,,	Niphad	22nd to 25th Oct. 1931.	..	1st week of January 1932.‡†	...	
			Kite slides.				
1	United Provinces	Agra	2nd Jan. 1932.	29th Jan. 1932 (45).	3rd week of February 1932.	...	
2	,,	,,	11th Jan. 1932.	...	...	2nd week of February 1932.	
3	,,	,,	24th Dec. 1931.	...	...	...	2nd week of February 1932.

* Material was collected by one of the assistants on that date.

† Very early spore shower. The very first slide exposed caught spores.

‡ Crop sown very late.

CHART III.

Showing the dates of earliest spore shower and the dates of rust appearance at different stations (1932-33).*

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
				Aeroscope slides.			
1	Punjab	Lyallpur	1st to 3rd Apl. 1933.	...	20th April 1933.	...	...
2	,,	,,	29th Mar. to 1st Apl. 1933.	...	...	Middle of April 1933.	...
3	,,	,,	7th to 11th Jan. 1933.	...	...	...	20th February 1933.
4	,,	Gurdaspur	13th to 17th Dec. 1932.	...	...	...	27th January 1933.
5	,,	Hoshiarpur	27th to 31st Jan. 1933.	...	Middle of March 1933.	...	...
6	,,	Rawalpindi	2nd to 5th Mar. 1933.	...	End of April 1933.	...	...
7	,,	,,	5th to 9th Mar. 1933.	...	...	2nd week of April 1933.	...
8	,,	,,	2nd to 5th Mar. 1933.	...	...	...	15th March 1933.
9	,,	Simla	21st to 25th Dec. 1932.	...	29th March 1933.	...	...
10	,,	,,	5th to 9th Oct. 1932.	...	...	9th March 1933.	...
11	,,	,,	18th to 21st Sep. 1932.	12th to 16th Oct. 1932 (50).	...	...	25th November 1932.
12	Sind	Sakrand	10th to 14th Feb. 1933.	...	20th March 1933.	...	...
13	United Provinces	Agra	31st Dec. to 3rd Jan. 1933.	...	5th March 1933.	...	...
14	,,	,,	4th to 7th Feb. 1933.	...	...	19th February 1933.	...

*Such stations, wherefrom no information was received about rust appearance or where no rust appeared, have been excluded from this list.

CHART III—*contd.*

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
				Aeroscope slides—contd.			
15	United Provinces	Agra . .	4th to 7th Feb. 1933.	...	...	...	15th February 1933.
16	,, .	Cawnpore †	16th to 19th Jan. 1933.	...	1st week of February 1933.	...	...
17	,, .	,, .	2nd to 6th Feb. 1933.	...	...	1st week of February 1933.	...
18	,, .	,, .	19th to 23rd Jan. 1933.	...	...	...	1st week of February 1933.
19	,, .	Gorakhpur .	19th to 22nd Nov. 1932.	...	10th January 1933.	...	...
20	,, .	,, .	14th to 19th Nov. 1932.	...	...	...	10th January 1933.
21	,, .	Nawabganj (Bareilly).	9th to 13th Jan. 1933.	19th to 25rd Feb. 1933 (90).	1st week of March 1933.	...	...
22	,, .	,, .	17th to 21st Jan. 1933.	...	...	1st week of February 1933.	...
23	,, .	,, .	17th to 21st Jan. 1933.	17th to 21st Jan. 1933 (35).	...	...	1st week of February 1933.
24	,, .	Almora .	2nd to 5th Jan. 1933.	21st to 26th Jan. 1933 (20).	12th March 1933.	...	...
25	,, .	,, .	5th to 8th Jan. 1933.	...	...	18th March 1933.	...
26	,, .	,, .	29th Oct. to 2nd Nov. 1932.	11th to 15th Nov. 1932 (37).	...	...	25th December 1932.
27	Bihar .	Pusa . .	14th to 17th Nov. 1932.	...	13th February 1933.	...	...

† The information received stated "Rust appeared in the first week of February". No rust was specified.

CHART III—*contd.*

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance		
					Black	Brown	Yellow
				Aeroscope slides—contd.			
28	Bihar .	Pusa . .	1st to 5th Dec. 1932.	...	...	29th December 1932.	...
29	,, .	,, . .	3rd to 7th Nov. 1932.	...	...	...	3rd February 1933.
30	,, .	Patna .	18th to 21st Jan. 1933.	18th to 21st Jan. 1933 (25).	6th March 1933.	...	...
31	,, .	,, .	9th to 13th Jan. 1933.	...	...	Last week of January 1933.	...
32	,, .	,, .	18th to 21st Jan. 1933.	...	...	...	Last week of January 1933.
33	,, .	Sabour (Bhagalpur).	26th to 29th Jan. 1933.	...	1st week of March 1933.	,	...
34	,, .	,, .	8th to 11th Jan. 1933.	16th to 19th Feb. 1933 (153).	...	2nd week of March 1933.	...
35	,, .	,, .	29th Jan. to 2nd Feb. 1933.	...	...	...	15th February 193
36	Rajputana	Ajmer .	27th Feb. to 2nd Mar. 1933.	...	11th March 1933.	...	...
37	Central Provinces	Jubbulpore.	11th to 15th Feb. 1933.	...	25th February 1933.	...	...
38	,, .	Khandwa .	30th Jan. to 2nd Feb. 1933.	...	14th March 1933.	...	...
39	Bombay-Deccan.	Dharwar .	15th to 18th Aug. 1932.	...	17th January 1933.	...	...
40	,, .	Poona .	20th to 24th Oct. 1932.	...	5th December 1932.	...	...
41	,, .	Niphad .	12th to 15th Aug. 1932.	...	11th December 1932.	...	...

CHART III—*contd.*

Serial No.	Province	Station	Dates of earliest spore shower	Dates on which a fairly large number of spores were caught at some of the stations well before rust appearance. Figures in brackets show the number of spores	Dates of rust appearance.		
					Black	Brown	Yellow
				Aeroscope slides—contd.			
42	Bombay-Deccan.	Arbhavi ‡ .	17th to 20th Oct. 1932.	...	2nd January 1933.	...	...
43	„ .	Dohad .	3rd to 7th Dec. 1932.	...	22nd March 1933.	...	...
44	„ .	Baroda .	12th to 15th Oct. 1932.	...	1st March 1933.	...	...
45	„ .	Mehsana .	7th to 10th Oct. 1932.	...	1st week of February 1933.	...	...
46	Hyderabad-Deccan.	Parbhani .	14th to 17th Sep. 1932.	...	19th January 1933.	...	...
47	Mysore State.	Bangalore .	8th to 12th Dec. 1932.	27th Jan. to 1st Feb. 1933 (172).	...	4th February 1933.	...
48	„ .	Mysore .	31st Dec. to 3rd Jan. 1933.	...	...	15th January 1933.	...
49	„ .	Chitaldroog §.	24th to 27th Dec. 1932.	...	End of November 1932.	...	...
50	Madras .	Coimbatore	15th to 18th July 1932.	...	27th December 1932.	...	...
				Kite slides.			
1	United Provinces	Agra .	14th Dec. 1932.	16th Feb. 1933 (28).	5th March 1933.	...	...
2	„ .	„ .	6th Jan. 1933.	...	...	19th February 1933.	...
3	„ .	„ .	17th Dec. 1932.	...	...	...	15th February 1933.

‡ Information of rust appearance was received from Masuguppi, which is 12 miles away from Arbhavi.

§ The information was received late in April after several reminders and may not be correct.

THE RELATIVE GROWTH RATE, THE CARBOHYDRATE CONTENTS AND THE YIELD OF THE RICE PLANT (*ORYZA SATIVA L.*) UNDER DIFFERENT TREATMENTS.

BY

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(With thirteen text-figures).

Introduction.

As the work done on the salt requirements of the rice plant and the effect of ammoniacal and nitrate nitrogen on its growth and yield are amply reviewed in previous contributions on the physiology of the rice plant by one of the authors [Dastur ; 1931, 1932, 1933] in this *Journal*, it is not considered necessary to do so here again.

As a result of the study on the intake of nitrogen by the rice plant, it has been recently suggested by Dastur and Malkani [1933] that a mixture of two fertilizers, nitrates and ammonium sulphate, will prove better than any one used singly. It has also been shown by various workers that by manuring the plants with ammonium sulphate in the early stages and with potassium nitrate in the later stages (at the flowering time) an increased yield of grain and straw is obtained. This view is based upon two conceptions, firstly the nitrates are absorbed in the later stages and secondly the assimilatory activity is greatest at the flowering stage. Both these conceptions are open to objections. The absorption of nitrates is fairly high before the flowering stage and the view that the nitrates are assimilated as soon as they are supplied to the soil at the time of the maximum assimilatory activity does not seem to be sound, as before the nitrates are assimilated and

incorporated into the substance of the plant, a long period must intervene and therefore it should be more profitable to supply the nitrates at an earlier stage than at the flowering stage or the milk stage. The manuring with nitrate at the latest stages of growth may produce some effect but that is not the maximum effect.

It was therefore undertaken to test the two points discussed above, *viz.*, the effect of mixture of nitrate and ammonium sulphate on the growth and yield of the rice plant as compared to any one used singly on equal nitrogen basis and secondly the best time of manuring the plants to obtain the maximum effect. So far as the authors are aware no attempt has been made previously to study the effect of a mixture of the two forms of nitrogen supplied in one dose to the rice plants on their growth and yield, and if the findings of Dastur and Malkani [1933] about the intake of nitrogen from ammonium salts and nitrates obtained from water culture experiments hold good in the case of plants grown in the soil, there should be a better growth of the rice plants as compared with the plants fertilized with either ammonium salts or with nitrates singly on an equal total nitrogen basis. It would be also profitable to investigate systematically the effect of manuring the plants with two forms of nitrogen, on their growth and yield at successive stages, as that would lead us to determine the period at which manuring produces maximum effect.

In order to study the effect of three forms of nitrogenous fertilizers on the growth of the rice plant, it would be necessary to determine the relative growth rate of the plant at successive stages of growth, as the relative growth rate would be the correct criterion to study the differences in growth made by the plant under different treatment.

It is also undertaken to determine the carbohydrate contents of the rice plants manured in these three different ways at each stage of growth, as it would then be possible to study the effect of ammoniacal and nitrate nitrogen on the assimilatory activity of the plants.

Investigation.

Rice seedlings of the Columba variety No. 42 were obtained from the Rice Research Station, Karjat, and were transplanted in July 1930 in the beds in the garden of the Institute. The usual care was taken to use uniform samples of soil for all beds and it was also ascertained that the soil did not lack in any of the necessary elements. Natural manure in equal doses was mixed with the soil. Arrangements for impounding water were made. Two rice seedlings were put together to make one plant at the time of transplantation in the beds at equal distances.

On the 1st August a rice plant was uprooted, the fresh weights of the leaves, culms and roots were noted. The volume of roots was measured by displacement of water in the measuring cylinder and the total area of leaves was recorded. The leaves, culms and roots were cut into small pieces and taken for extraction of carbohydrates. In all cases of extraction of carbohydrates leaves were taken for extraction separately from the culms and roots in separate round-bottom flasks.

On the 1st August one group of plants was manured with potassium nitrate, 2nd group of plants was manured with ammonium sulphate and a third group of plants was manured with a mixture of potassium nitrate and ammonium sulphate. The doses of fertilizers used were calculated on equal nitrogen basis so that the total nitrogen supplied to each group of plants was the same in three cases.

On the 15th August one plant was uprooted from each of the three groups and the fresh weights of leaves, roots and culms, the volume of the roots and the total area of the leaves, were recorded, and the leaves, culms and roots were cut into small pieces and taken for the extraction and estimation of carbohydrates. Side by side a plant from the unmanured bed was taken and similarly treated. On the 15th August three groups of plants, not manured before, were separately manured with potassium nitrate, ammonium sulphate and a mixture of the two as on the 1st August.

On the 1st September one plant from each of the three groups of plants manured on the 1st August and one plant from each of the three groups manured on the 15th August were uprooted and treated as before. A plant from the unmanured group was also taken and similar values obtained.

On the 1st September the 3rd series of three groups of plants, not manured before, were separately manured with the three fertilizers as on previous occasions.

On the 15th September one plant from each of the nine groups manured in three different ways on the three successive dates were similarly treated. One unmanured plant was also taken for various analyses.

The same procedure was again followed and three groups of plants were manured on the 15th September and three groups on the 1st October and the plants were analysed as before on the 1st October and the 15th October.

On the 1st October 12 *plus* one plants had to be analysed separately and on the 15th October the number of plants analysed similarly was 15 *plus* one.

The plants were not manured after the 1st October (*i.e.* on the 15 October) as by the 1st of November the plants would be more or less dead.

The fifteen groups of manured plants on five subsequent dates and the totally unmanured plants were harvested on the 23rd November and the weights of grain and air-dried straw recorded. No other records could be kept as the plants were dead.

EXTRACTION AND DETERMINATION OF CARBOHYDRATES.

The carbohydrates of the leaves, culms and roots are extracted and quantitatively determined according to the method recently described by Dastur and Samant [1933] in this *Journal*. The reducing sugars, cane sugar and starch were determined separately in each group of plants both manured and unmanured.

The number of carbohydrate analyses to be made increased at every subsequent date in the arithmetical progression from 1 to 3, 6, 9, 12, 15 and *plus* one. It was found difficult to manage the same in one day. For this reason a day before and a day after the dates mentioned above were utilized for those observations. The total number of extraction apparatus had to be increased to twenty-four or more and the arrangement had to be made for redistilling the large quantities of alcohol used. Two water baths holding fourteen evaporating dishes at the same time had to be used for the evaporation purpose and an incubator, holding twelve 1000-c.c. beakers, was used for the hydrolysis of starch. The estimations of carbohydrates according to the methods described above were begun in November and finished in April. Two months were taken to calculate the results of the same year.

In the following Tables (I to VI) the fresh weight of the leaves, of the whole plant, volume of the roots, total leaf area, the total water content and the residual dry weight of the unmanured plants and of the plants manured with three fertilizers on successive stages of growth are given.

Taking the unmanured plant first (in Tables I and V) it is seen that the fresh weight of leaves increases from 1·22 grams to 75·2 grams and fresh weight of the whole plant increases from 5·219 grams to 346·5 grams. The volume of roots rises from 1·5 c.c. to 60 c.c. and the total leaf area rises from 108·12 square centimeters to 5048·4 sq. cm. The residual dry weights after extraction of carbohydrates increase from 0·6515 gram to 69·48 grams. The term residual dry weight means dry weight *minus* the soluble matter and carbohydrates.

In the case of manured plants it will be noticed that the rise in the fresh weights of the plants, in leaf areas, residual dry weights and the volumes of the roots are largest in the plants manured with any one of the three fertilizers on the 15th August. Of the plants manured with each one of the three fertilizers on the 15th August, the increase in fresh and residual dry weights, in leaf area and in the volume of roots are greatest in plants manured with the mixture. In plants manured with a mixture of the two fertilizers the fresh weight of the plant rises from 5·219 grams to 532·6 grams, the residual dry weight of the plant rises from 0·6515 grams to 101·68 grams on the 15th October, the total leaf area rises from 108·12 sq. cm. to 8680 sq. cm. and the volume of the roots rises from 1·5 c.c. to 72·5 c.c. (in Tables I and V).

The results indicate that the maximum increase in fresh and dry weights, in leaf area and in the volume of roots takes place when the plants are manured with the mixture of potassium nitrate and ammonium sulphate. Secondly the plants manured on the 15th August show better growth than the plants manured at any other stage of growth.

In Table VI the dry weights of straw and grain of the plants harvested on 23rd November are given. The straw and grain are dried in air. Here also the maximum yield of straw and grain are obtained in plants manured with the mixture. The yield of grain and straw is the largest in plants manured on the 15th August in potassium nitrate series, ammonium sulphate series and the mixture series.

The ratios of the weights of straw to the weights of grain are 2·8 : 1 for the unmanured plants, 2·6 : 1 for plants manured with potassium nitrate, 2·4 : 1 for plants manured with ammonium sulphate and 2 : 1 for the plants manured with the mixture. Thus the manuring with the mixture of potassium nitrate and ammonium sulphate results in better reproduction as compared to vegetative growth in plants manured either with potassium nitrate or ammonium sulphate.

TABLE I.

Date of manuring.	Nature of manure	Fresh wt. of leaves in gms.	Total fresh wt. of the plant (Leaves + culms + roots) in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Residual dry wt. of the plant in gms.
			1st August 1930.				
	Unmanured	1·22	5·219	1·5	108·12	4·5437	0·6515
			15th August 1930.				
	Unmanured	3·855	19·315	3·8	281·10	17·528	1·64
1st August	KNO_3	5·570	25·390	4·0	370·71	23·5019	2·63
1st ,,	$(NH_4)_2SO_4$	7·150	29·200	4·6	589·35	26·0281	2·98
1st ,,	$KNO_3 + (NH_4)_2SO_4$	8·110	32·740	5·2	635·58	29·0331	3·38

TABLE II.

Date of manuring	Nature of manure	Fresh wt. of leaves in gms.	Total fresh wt. of the plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Residual dry wt. of the plants in gms.
				1st September			
	Unmanured . .	14·18	62·85	10·00	884·52	56·23	6·62
1st August .	KNO_3 . . .	15·72	75·42	10·00	1054·50	67·36	8·06
15th ,, .	,, . . .	17·04	78·19	10·48	1306·40	69·09	9·09
1st ,, .	$(NH_4)_2SO_4$ . . .	17·45	78·92	10·50	1294·60	69·33	8·97
15th ,, .	,, . . .	18·50	86·93	11·52	1393·50	76·99	9·94
1st ,, .	$KNO_3+(NH_4)_2SO_4$.	20·30	88·52	13·58	1475·65	78·68	9·83
15th ,, .	,, .	25·19	105·94	15·50	1895·00	93·27	12·13

TABLE III.

Date of manuring	Nature of manure	Fresh wt. of leaves in gms.	Total fresh wt. of the plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Residual dry wt. of the plants in gms.
				15th September			
	Unmanured . .	39·48	180·47	26·40	2016·4	161·98	18·31
1st August .	KNO_3 . . .	45·50	205·20	26·50	2279·4	183·13	21·07
15th ,, .	,, . . .	48·20	216·10	27·55	2483·7	191·37	24·73
1st September .	,, . . .	47·10	218·70	29·50	2406·6	193·98	24·73
1st August .	$(NH_4)_2SO_4$ . .	51·15	229·50	28·00	2613·4	203·62	25·78
15th ,, .	,, . .	52·88	235·30	28·40	2817·5	208·89	26·41
1st September .	,, . .	50·30	224·95	28·00	2600·2	199·51	25·44
1st August .	$KNO_3+(NH_4)_2SO_4$.	58·65	255·10	32·60	2905·5	228·25	26·87
15th ,, .	,, .	66·82	278·42	40·50	3716·5	247·48	30·94
1st September .	,, .	58·30	259·30	32·00	3013·7	231·80	28·50

TABLE IV.

Date of manuring	Nature of manure	Fresh wt. of leaves in gms.	Total fresh wt. of the plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Residual dry wt. of the plant in gms.
				1st October			
	Unmanured . .	67·50	297·50	50·0	4600·0	251·44	45·96
1st August .	KNO_3 . . .	82·20	365·50	59·5	5796·2	307·39	58·11
15th ,, .	,, . . .	85·40	379·80	61·0	5817·3	319·00	61·80
1st September .	,, . . .	86·42	386·82	62·0	5868·2	325·50	61·32
15th ,,	,, . . .	86·40	388·70	63·0	5826·9	327·30	61·40
1st August .	$(NH_4)_2SO_4$ . .	86·81	380·43	60·5	5947·0	319·44	60·99
15th ,, .	,, . . .	91·40	403·51	65·5	6948·0	339·42	64·09
1st September .	,, . . .	88·22	390·02	62·8	6272·5	328·66	61·36
15th ,,	,, . . .	89·70	402·60	66·4	6782·3	338·84	63·76
1st August .	$KNO_3+(NH_4)_2SO_4$.	109·20	461·80	66·9	8030·0	393·59	68·17
15th ,, .	,, .	114·50	483·40	67·5	8297·5	396·50	68·81
1st September .	,, .	99·80	442·23	66·5	7662·4	374·92	67·31
15th ,,	,, .	102·00	453·70	66·2	7702·8	385·89	67·81

TABLE V.

Date of manuring	Nature of manure	Fresh wt. of leaves in gms.	Total fresh wt. of the plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Residual dry wt. of the plant in gms.
				15th October			
	Unmanured . .	75·20	346·5	60·0	5048·4	277·02	69·48
1st August .	KNO_3 . . .	95·10	430·0	64·0	7269·5	346·23	83·77
15th ,, .	,, . . .	97·60	450·1	67·5	7412·4	363·72	86·05
1st September .	,, . . .	90·20	414·6	63·5	6756·8	334·81	79·79
15th ,, .	,, . . .	87·60	404·6	60·5	5972·3	326·16	78·44
1st October .	,, . . .	80·40	360·7	59·8	5382·6	289·22	71·48
1st August .	$(NH_4)_2SO_4$ . . .	96·80	445·1	67·5	7441·4	359·18	85·92
15th ,, .	,, . . .	98·20	454·8	68·2	7562·8	366·93	87·90
1st September .	,, . . .	93·20	427·7	63·5	7032·6	345·02	82·68
15th ,, .	,, . . .	90·40	421·2	63·5	6802·2	340·22	80·98
1st October .	,, . . .	83·00	385·4	62·8	5836·9	310·62	74·76
1st August .	$KNO_3+(NH_4)_2SO_4$.	120·10	513·1	70·0	8492·4	414·49	98·61
15th ,, .	,, .	123·20	532·6	72·5	8680·0	430·92	101·68
1st September .	,, .	114·80	491·0	67·5	8422·2	396·43	94·59
15th ,, .	,, .	112·20	483·5	67·5	8401·9	389·77	93·73
1st October .	,, .	87·80	401·4	63·2	6120·0	323·68	77·72

TABLE VI.

Date of manuring	Nature of manure	Weight of straw in gms.	Weight of grain in gms.	Total weight	Ratio of straw to grain
			23rd November		
	Unmanured . .	75·0	26·70	101·70	2·8:1
1st August . .	KNO_3 . . .	83·0	31·11	114·11	2·6:1
15th ,, . .	,, . . .	85·0	32·42	117·42	2·6:1
1st September .	,, . . .	80·5	30·20	110·50	2·6:1
15th ,, .	,, . . .	82·5	32·10	114·60	2·6:1
1st October .	,, . . .	76·0	27·21	103·21	2·6:1
1st August . .	$(NH_4)_2SO_4$ . . .	89·0	37·21	126·21	2·4:1
15th ,, . .	,, . . .	91·0	39·82	130·82	2·3:1
1st September .	,, . . .	86·5	35·93	122·43	2·4:1
15th ,, .	,, . . .	88·0	36·42	124·42	2·4:1
1st October .	,, . . .	76·0	30·60	106·60	2·5:1
1st August .	$KNO_3+(NH_4)_2SO_4$.	100·0	48·21	148·21	2:1
15th ,, . .	,, .	104·0	52·52	156·52	2:1
1st September .	,, .	93·0	46·11	139·11	2:1
15th ,, .	,, .	95·5	47·40	142·40	2:1
1st October .	,, .	78·0	32·82	110·82	2·3:1

The above increase in dry weights of the rice plant, when manured in three different ways at different periods, should be mathematically interpreted, as the mere differences in the dry weights do not convey any meaning. It is necessary to determine the ratio of increase in dry weights produced at the end of a period to the actual dry weight present at the beginning of the period, *i.e.*, the relative growth rate of the plant in each period must be measured under each treatment. The relative growth rate is here measured by the formula $W_1=W_0e.\ rt$ given by Blackman [1919, 1920] when W_0=dry weight of the plant at the beginning of the period t, W_1=dry weight of the plant at the end of the period t, r is the rate of increase in dry weight and e is the logarithmic base.

Simplifying the above formula—

$$\text{Log}_e W_1 \ldots \text{Log}_e W_0 + rt.$$

$$\textit{i.e.}, r \ldots \frac{\text{Log}_e W_1 - \text{Log}_e W_0}{t}$$

$\therefore$ relative growth rate (R. G. R.)=$\text{Log}_e W_1 - \text{Log}_e W_0$, when t=any arbitrary period chosen which is here a fortnight.

The relative growth rate of the whole plant unmanured and manured in three different ways at different dates was calculated. The following tables (Tables VII, VIII & IX) give the values of the relative growth rate calculated according to the above formula.

TABLE VII.

Rate of growth values of unmanured plants and of those manured with KNO_3.

Date of manuring	1st Aug. to 15th Aug.	15th Aug. to 1st Sep.	1st Sep. to 15th Sep.	15th Sep. to 1st Oct.	1st Oct. to 15th Oct.	15th Oct. to 15th Nov.
Unmanured	0·94	1·40	1·03	0·91	0·45	0·14
Manured with KNO_3 1st Aug.	1·41	1·13	0·97	1·02	0·40	0·10
„ „ „ 15th „	0·94	1·72	1·01	0·90	0·38	0·10
„ „ „ 1st Sep.	0·94	1·40	1·33	0·91	0·30	0·11
„ „ „ 15th „	0·94	1·40	1·03	1·21	0·28	0·13
„ „ „ 1st Oct.	0·94	1·40	1·03	0·91	0·47	0·13

TABLE VIII.

Rate of growth of the plants manured with $(NH_4)_2SO_4$.

1st August	1·53	1·10	1·08	0·86	0·27	0·14
15th „	0·94	1·81	0·99	0·88	0·35	0·14
1st September . . .	0·94	1·40	1·36	0·88	0·33	0·14
15th „ . . .	0·94	1·40	1·03	1·24	0·27	0·16
1st October . . .	0·94	1·40	1·03	0·91	0·52	0·13

TABLE IX.

Rate of growth of plants manured with mixture.

1st August	1·67	1·07	1·02	0·94	0·39	0·15
15th „	0·94	2·02	0·94	0·79	0·43	0·16
1st September . . .	0·94	1·40	1·47	0·86	0·37	0·14
15th „ . . .	0·94	1·40	1·03	1·32	0·35	0·15
1st October . . .	0·94	1·40	1·03	0·91	0·56	0·13

It will be seen that the relative growth rates of the unmanured plants as well as of the plants manured with any one of the three fertilizers are highest during the period of 15th August to 1st September with the exception of plants manured on 1st August. Similarly the plants manured with the mixture of potassium nitrate and ammonium sulphate have higher relative growth rates than the relative growth rates of the plants manured with either potassium nitrateor ammonium sulphate on the corresponding dates.

The graphs illustrating the relative growth rates of the unmanured and of the manured plants in three ways on the five successive dates are given in Figs. 1—5.

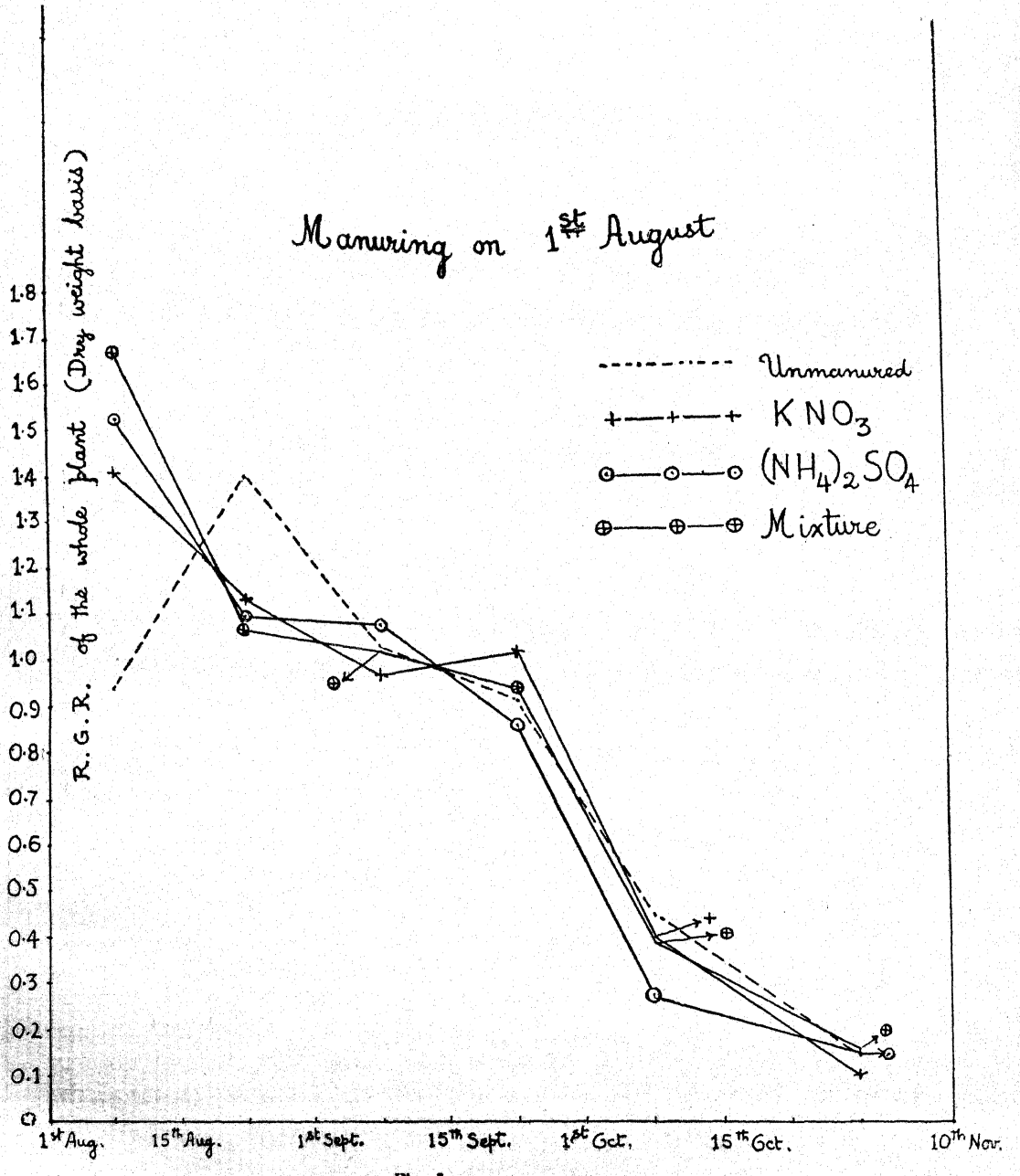


Fig. 1.

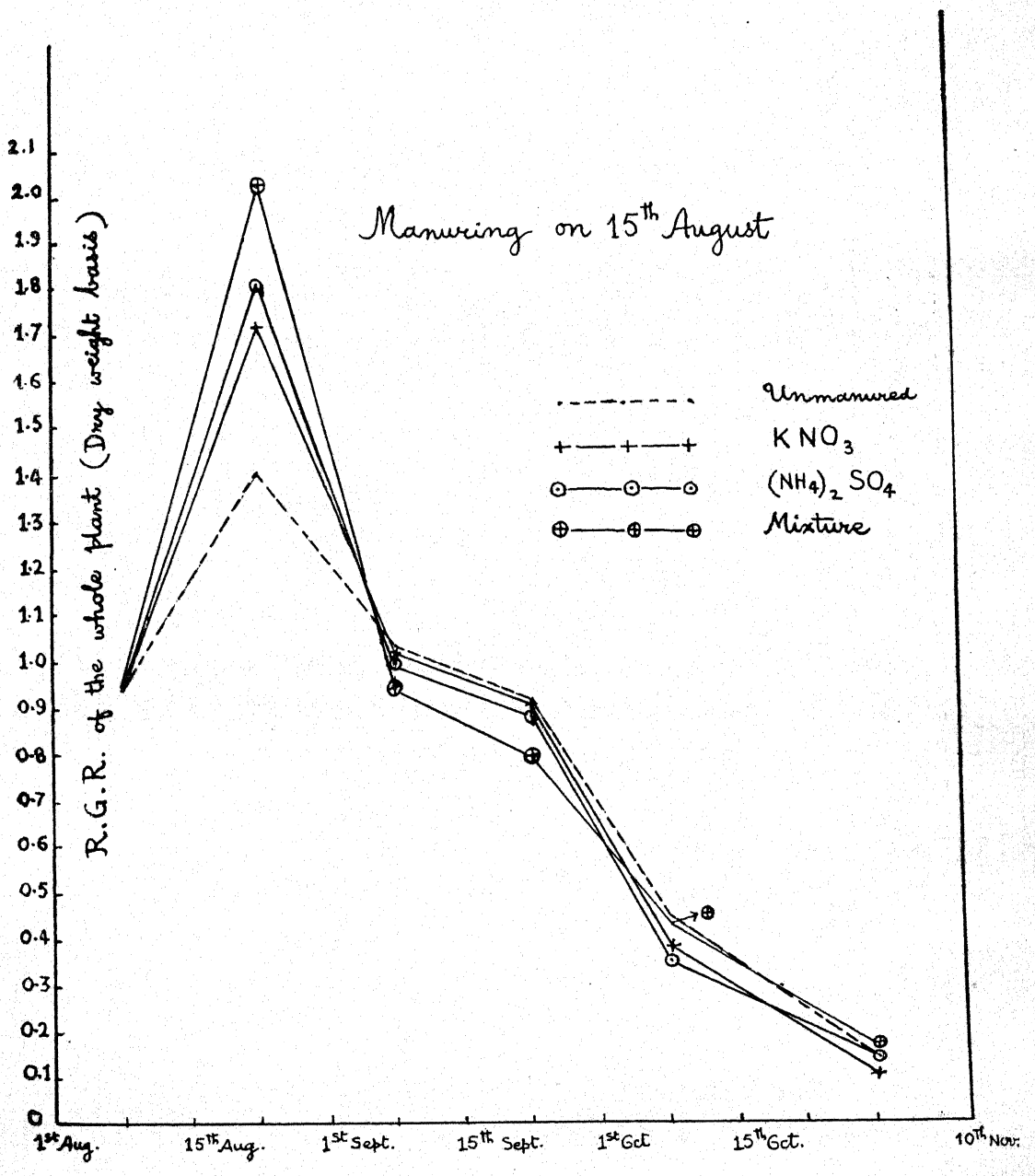

Manuring on 15th August
R.G.R. of the whole plant (Dry weight basis)
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Unmanured
KNO3
(NH4)2 SO4
Mixture
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.
10th Nov.

Fig. 2.

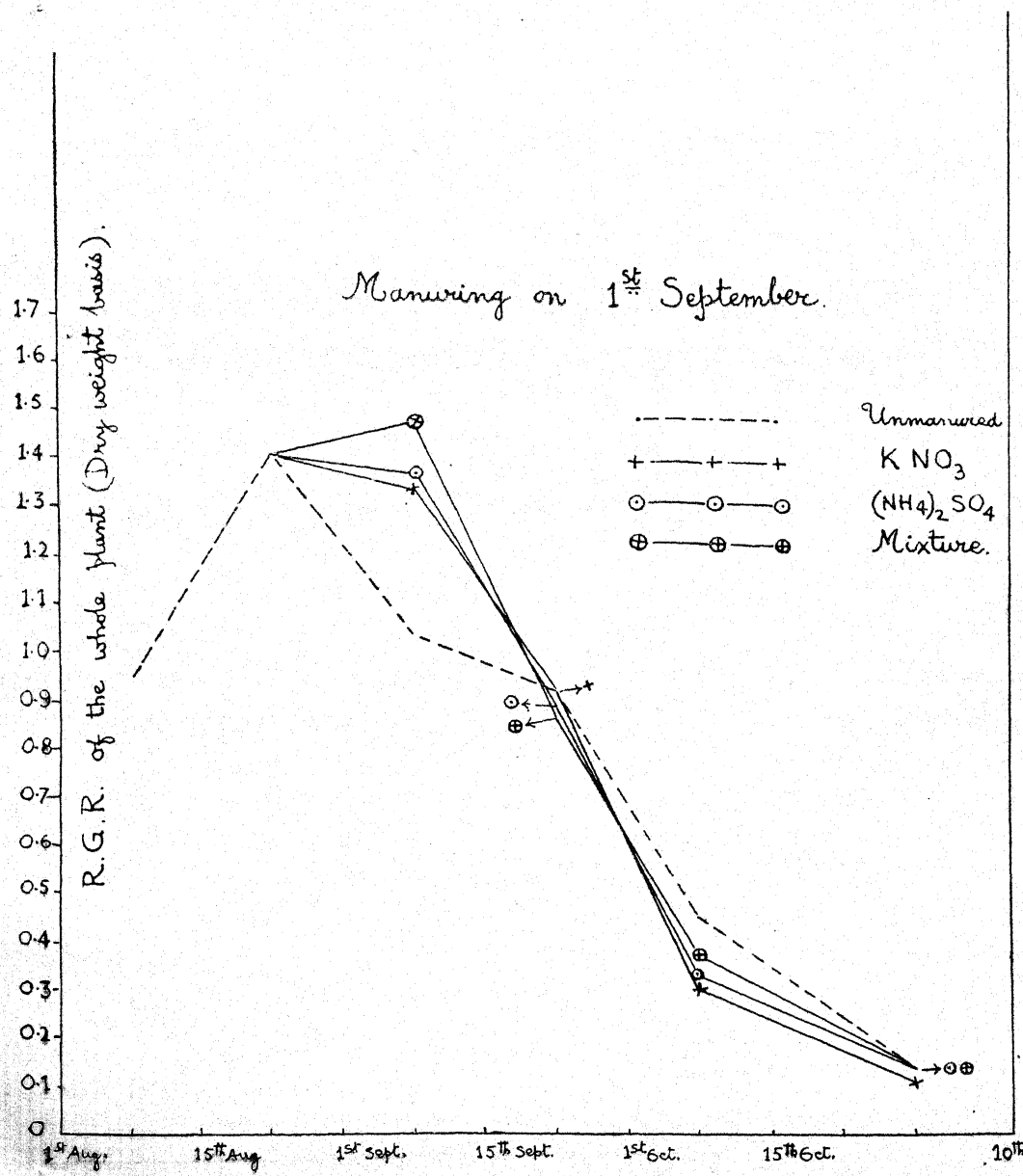

Manuring on 1st September.
R.G.R. of the whole plant (Dry weight basis).
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
Unmanured
KNO3
(NH4)2SO4
Mixture.
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.
10th Nov.

Fig. 3.

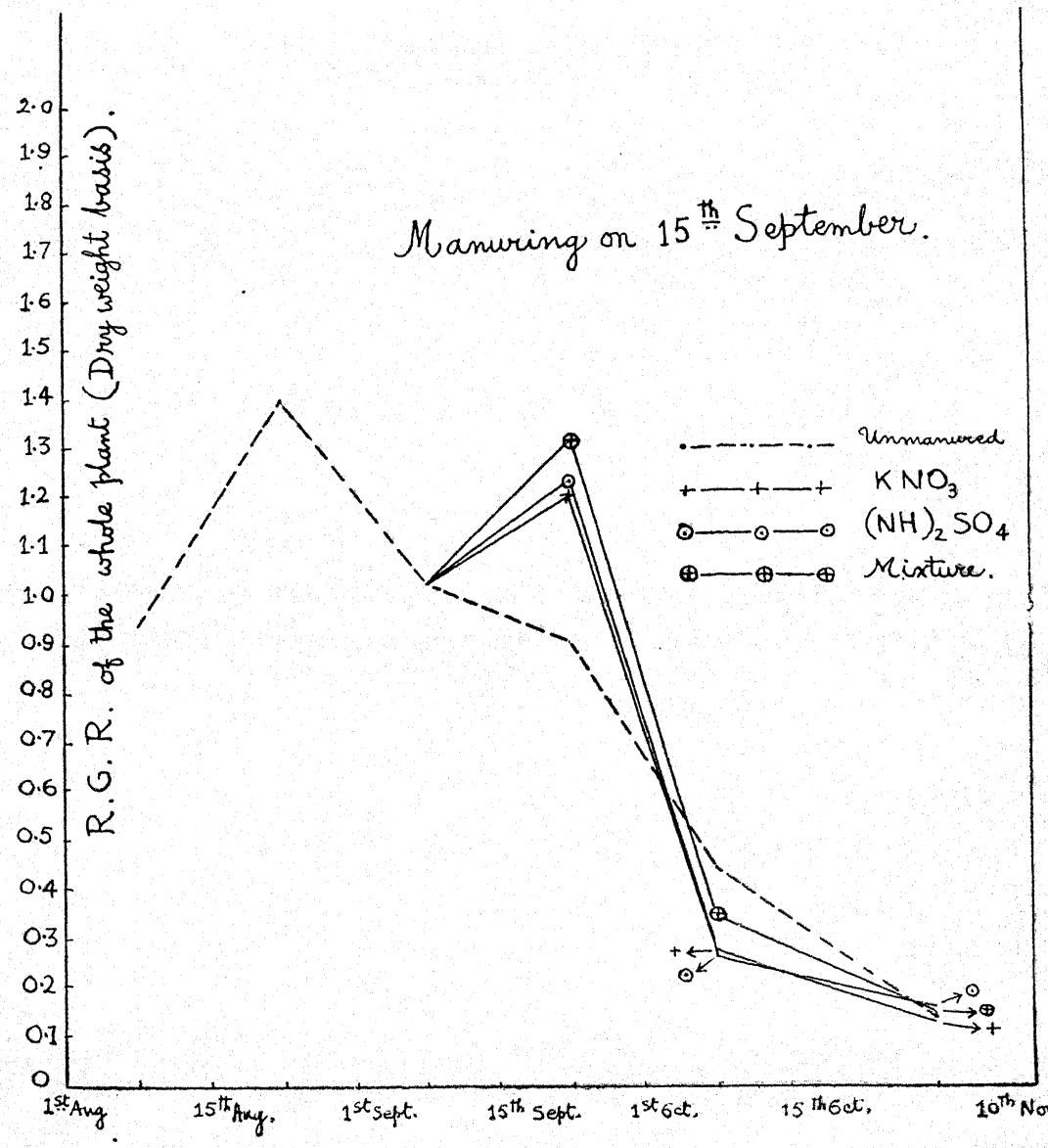

Manuring on 15th September.
R. G. R. of the whole plant (Dry weight basis).
2·0
1·9
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
Unmanured
KNO3
(NH)2 SO4
Mixture.
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.
10th Nov.

Fig. 4.

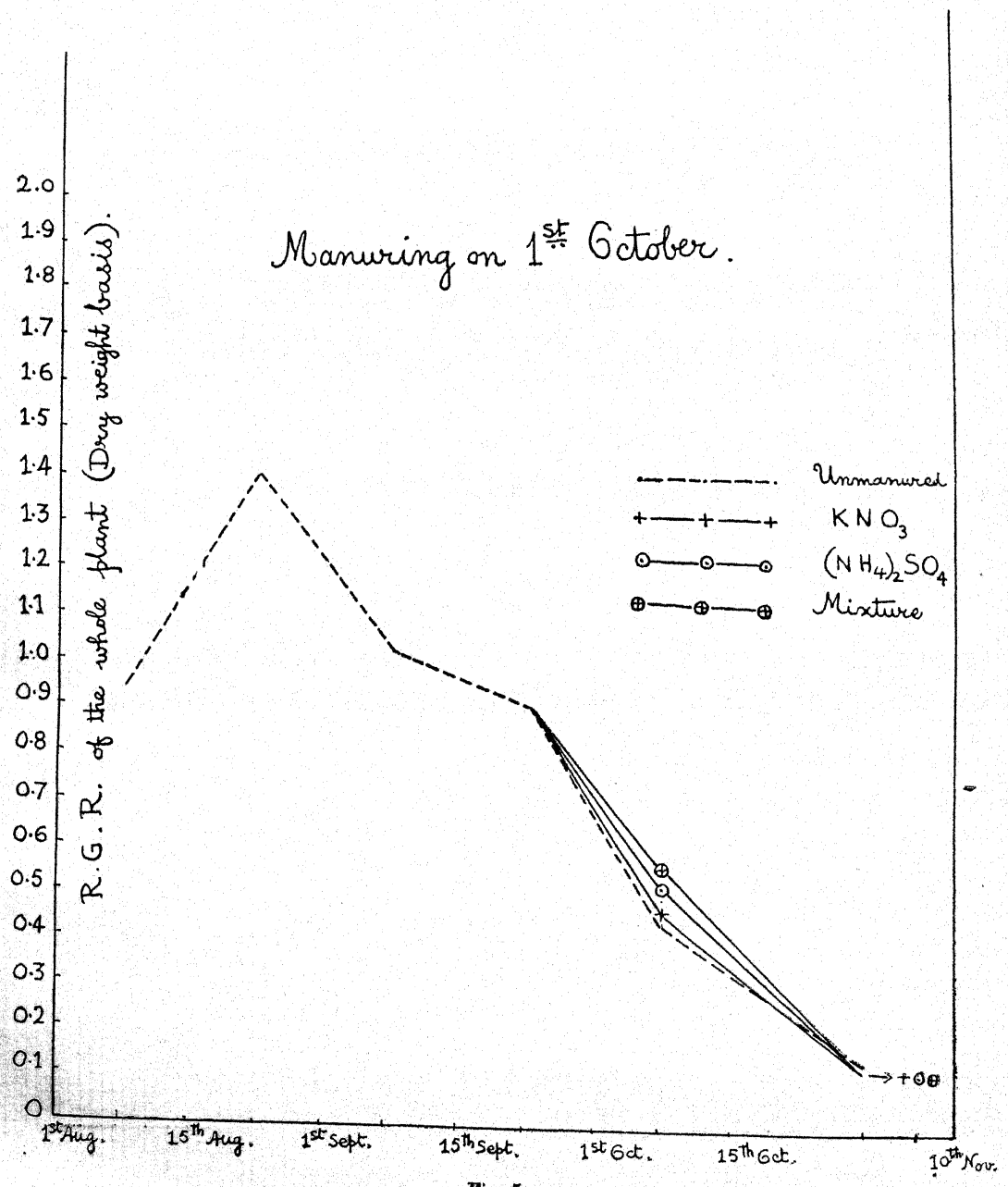

Manuring on 1st October.
R.G.R. of the whole plant (Dry weight basis).
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Unmanured
KNO3
(NH4)2SO4
Mixture
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.
10th Nov.

Fig. 5.

The relative growth rate of the unmanured plant calculated on fresh weight basis is given in Fig. 6, to show that the nature of the curve is the same as the relative growth rate curve on the dry weight basis.

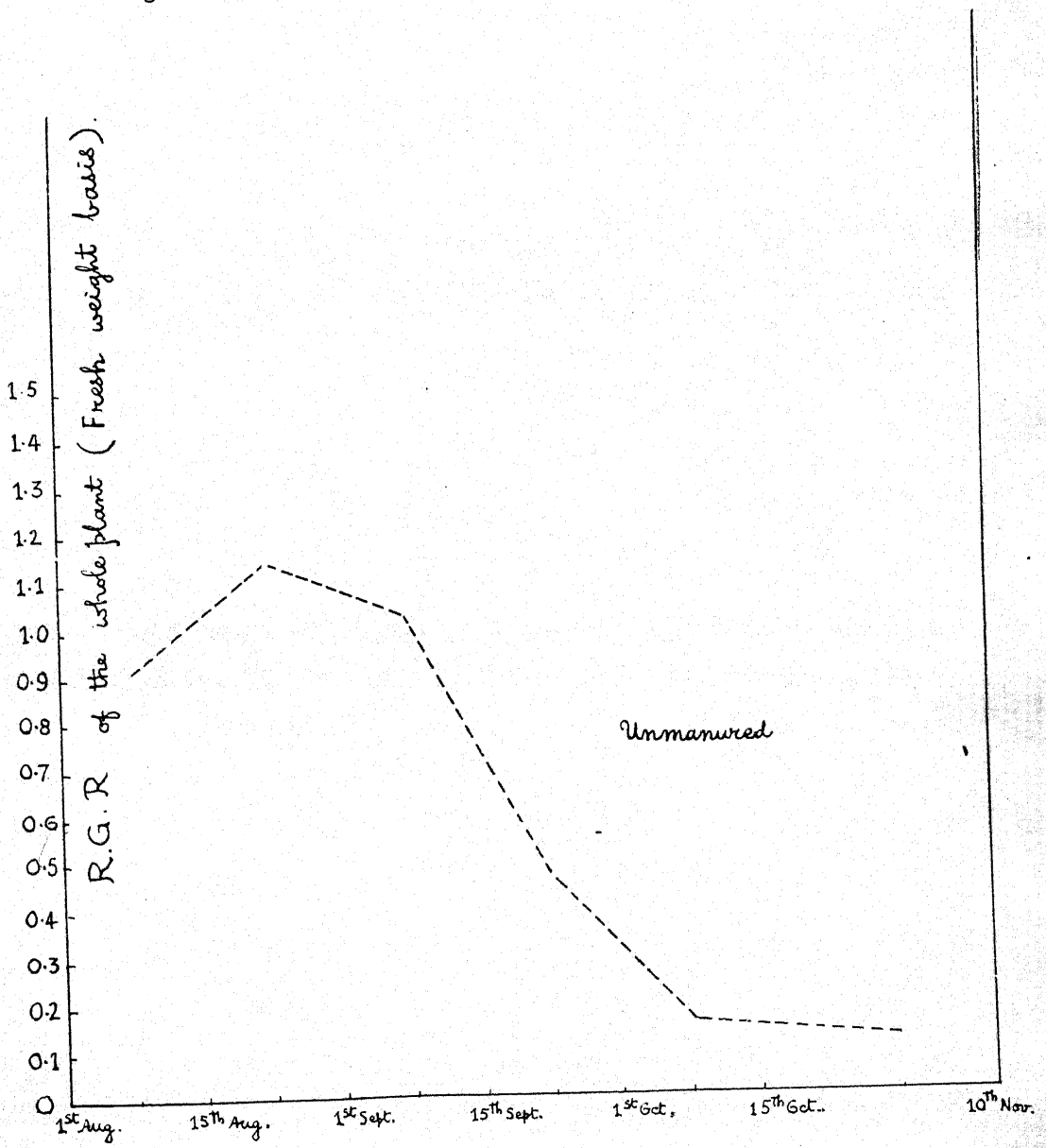


Fig. 6.

The relative growth rates of the leaves and culms and roots on dry weight basis are calculated and similar results, as given above for the whole plant, are obtained. In Figs. 7 and 8 the relative growth rate of the leaves of unmanured plant and of the plants manured in three ways on the 1st and 15th August are

given. When the plants are manured on the 1st August the highest relative growth rates of the whole plant as well as of the leaves are reached on the period 1st August to 15th August and not in the period of 15th August to 1st September.

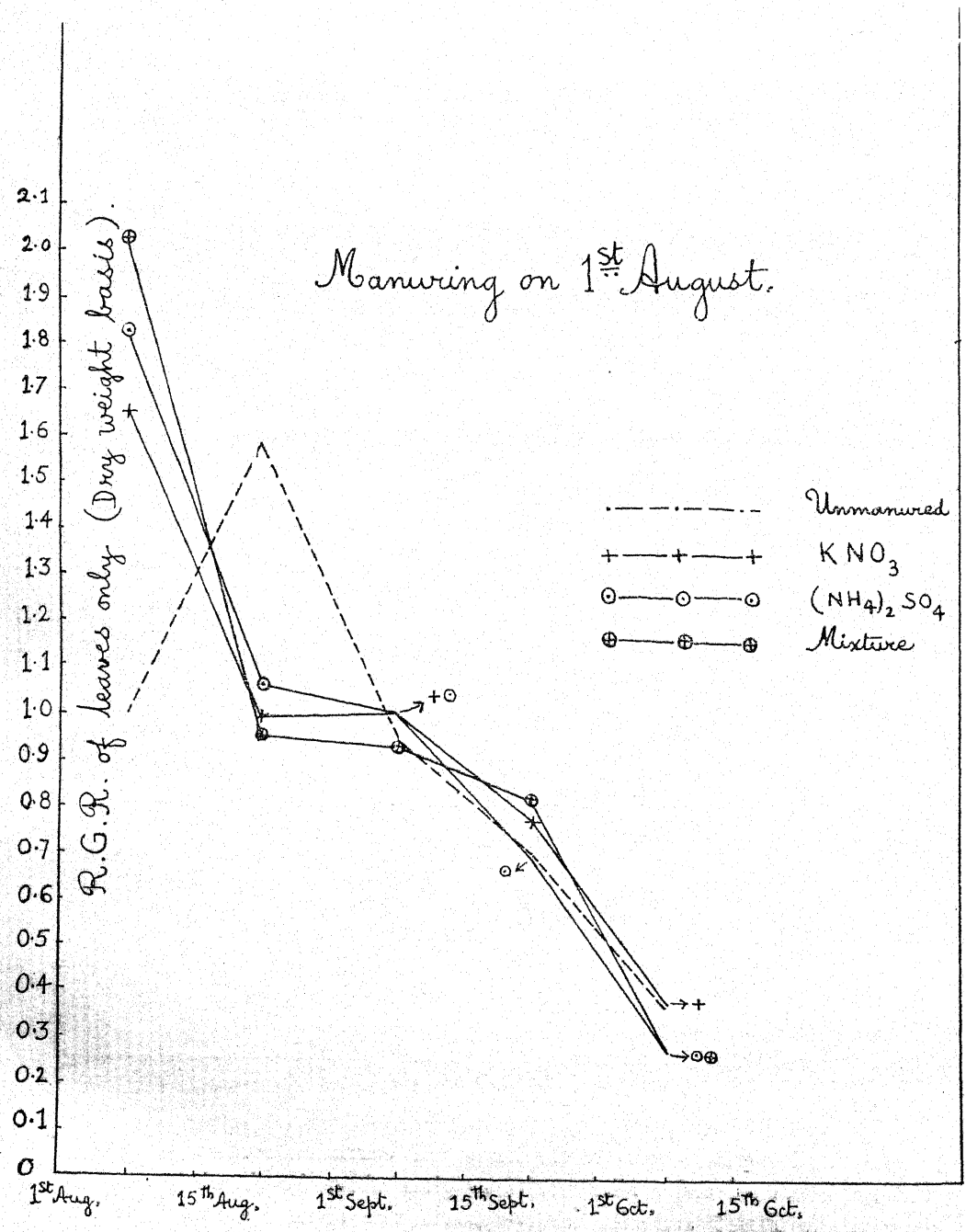
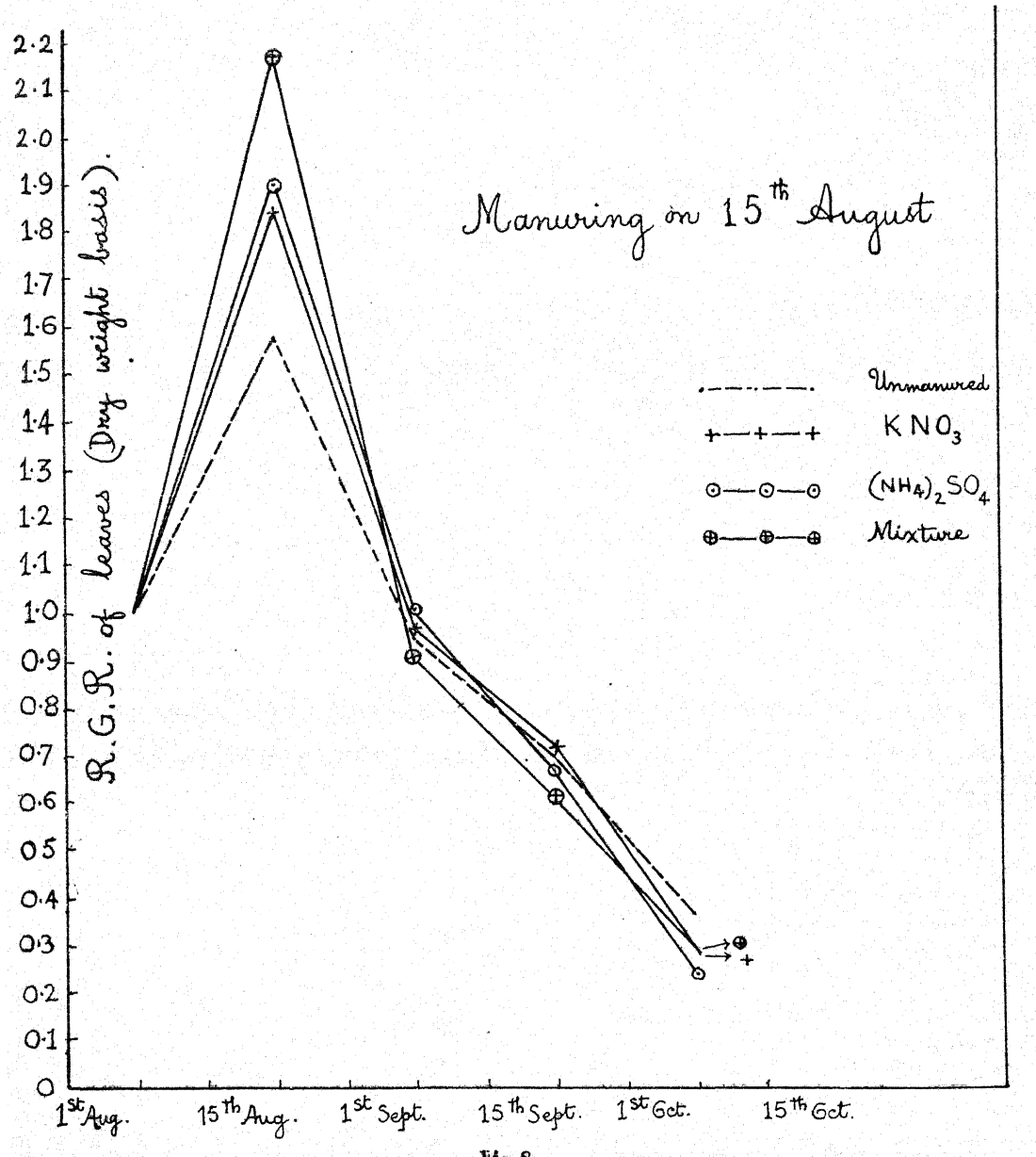


Fig. 7.


R.G.R. of leaves (Dry weight basis).
2·2
2·1
2·0
1·9
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.
Manuring on 15th August
Unmanured
KNO3
(NH4)2SO4
Mixture

Figs 8.

Similarly, the leaf area ratio, *i.e.*, the total area of the leaves per unit dry weight, is determined as the leaves are the chief organs manufacturing the food materials, which build up the body of the plant. The leaf area ratios are given in Tables X, XI and XII.

TABLE X.

Leaf area ratio of the unmanured plant and of the plants manured with KNO_3.

Date of manuring	1st August	15th August	1st September	15th September	1st October	15th October
Unmanured .	159·9	162·2	126·3	102·7	93·9	65·9
1st August .	159·9	133·6	122·9	108·4	93.2	78·2
15th „ .	159·9	162·2	135·2	93·0	89·3	79·3
1st September .	159·9	162·2	126·3	91·1	89·6	76·3
15th „ .	159·9	162·2	126·3	102·7	88·8	68·7
1st October .	159·9	162·2	126·3	102·7	93·9	68·2

TABLE XI.

Leaf area ratio of the plants manured with $(NH_4)_2SO_4$.

1st August .	159·9	187·8	137·0	94·2	90·5	77·9
15th „ .	159·9	162·2	131·4	99·0	100·6	77·3
1st September .	159·9	162·2	126·3	95·0	95·1	76·4
15th „ .	159·9	162·2	126·3	102·7	99·3	75·7
1st October .	159·9	162·2	126·3	102·7	93·9	70·6

TABLE XII.

Leaf area ratio of the plants manured with mixture.

1st August .	159·9	176·0	140·0	100·0	111·1	76·2
15th „ .	159·9	162·0	145·0	111·0	108·5	76·2
1st September .	159·9	162·2	126·3	97·9	105·2	79·6
15th „ .	159·9	162·2	126·3	102·7	105·0	83·6
1st October .	159·9	162·2	126·3	102·7	93·9	71·1

The results of the leaf area ratio disclose interesting features. The leaf area ratio is higher on the 15th August when the plants are manured with ammonium sulphate on the 1st August than when the plants are manured with the mixture. If the

results are studied for the manuring done on other dates there is a greater value of leaf area ratio in the plants manured with the mixture on the subsequent dates.

The graphs illustrating the leaf area ratios of the unmanured plant and of the plants manured in the three different ways on the five successive dates are given in figs. 9—13.

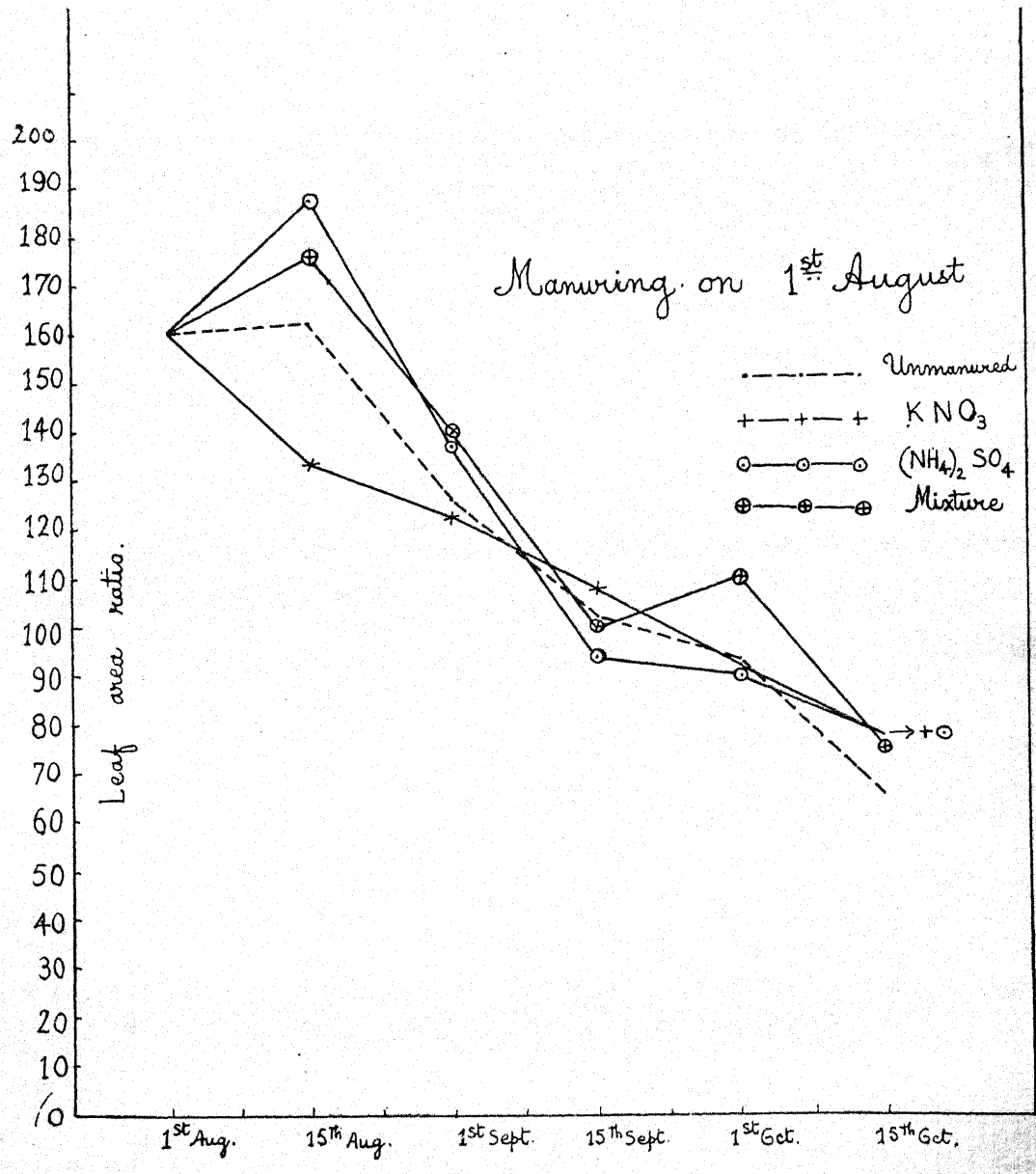


Fig. 9.

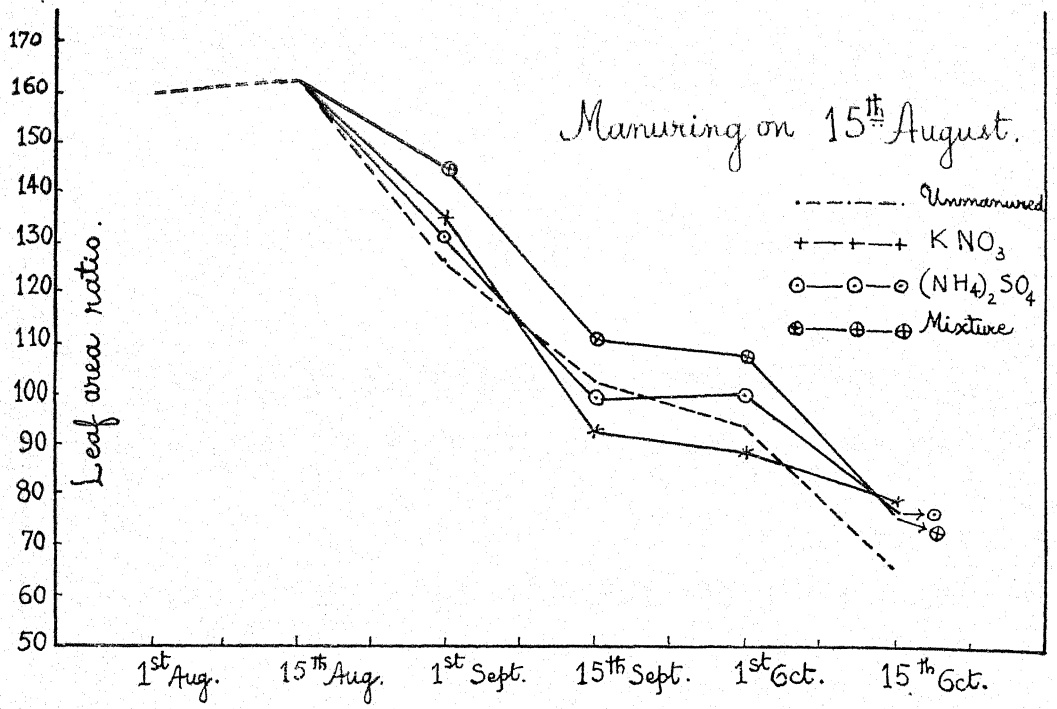

Manuring on 15th August.
Unmanured
KNO3
(NH4)2SO4
Mixture
Leaf area ratio.
170
160
150
140
130
120
110
100
90
80
70
60
50
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.

Fig. 10.

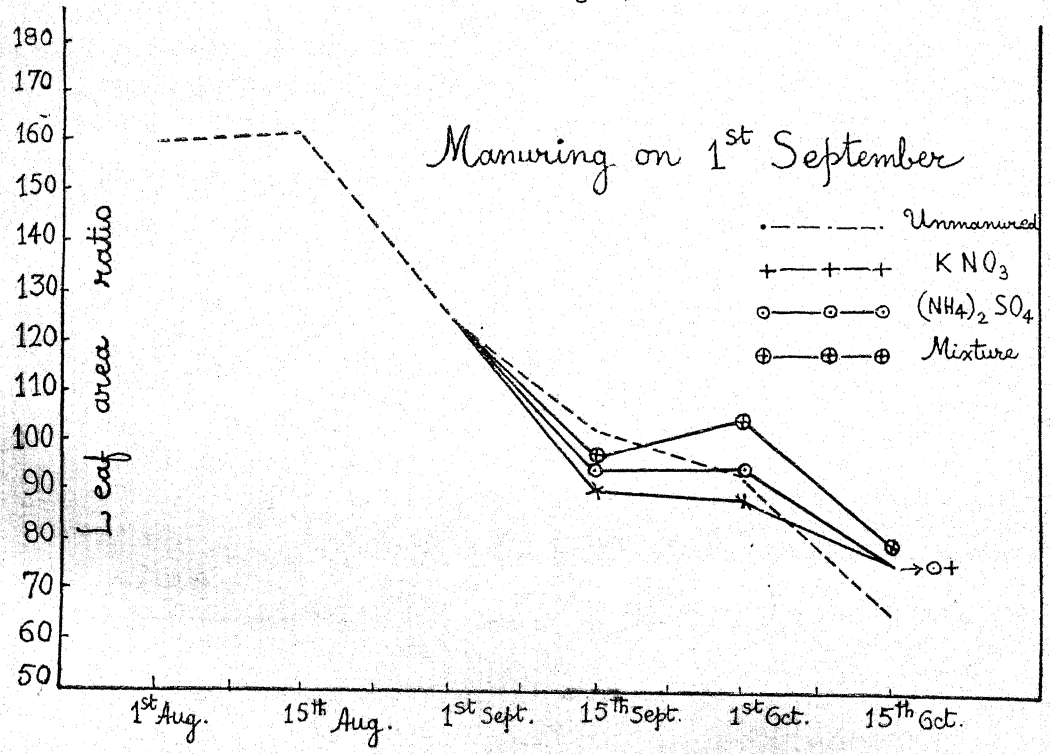

Manuring on 1st September
Unmanured
KNO3
(NH4)2SO4
Mixture
Leaf area ratio
180
170
160
150
140
130
120
110
100
90
80
70
60
50
1st Aug.
15th Aug.
1st Sept.
15th Sept.
1st Oct.
15th Oct.

Fig. 11.

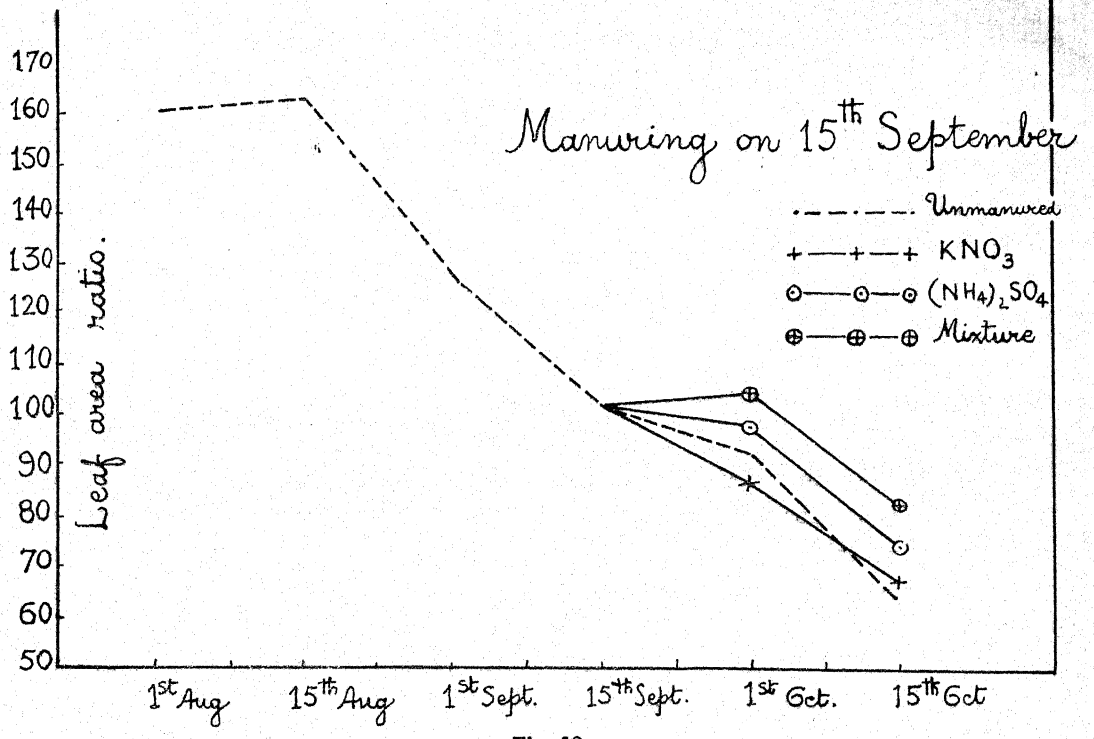

Manuring on 15th September
Unmanured
KNO3
(NH4)2SO4
Mixture
Leaf area ratio.
170
160
150
140
130
120
110
100
90
80
70
60
50
1st Aug
15th Aug
1st Sept.
15th Sept.
1st Oct.
15th Oct

Fig. 12.

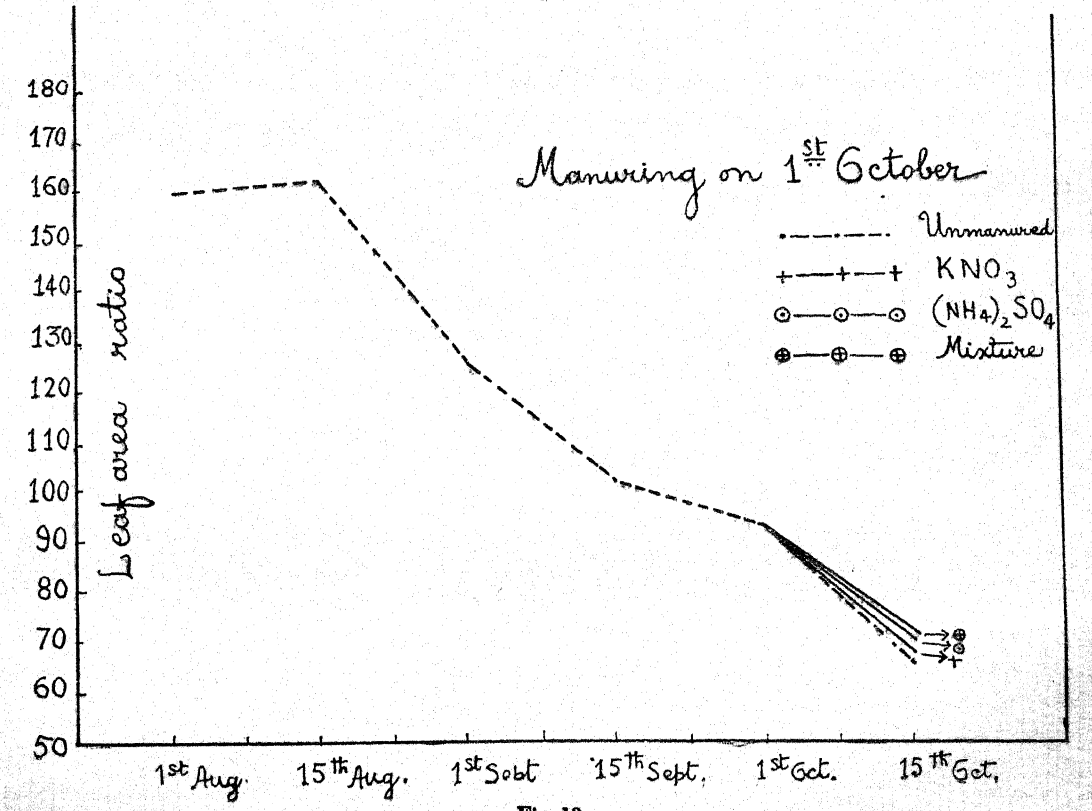

Manuring on 1st October
Unmanured
KNO3
(NH4)2SO4
Mixture
Leaf area ratio
180
170
160
150
140
130
120
110
100
90
80
70
60
50
1st Aug.
15th Aug.
1st Sept
15th Sept.
1st Oct.
15th Oct.

Fig. 13.

The leaf area per unit dry weight reaches its highest point when the plants are manured on the 1st August by ammonium sulphate. The mixture of potassium nitrate and ammonium sulphate has lesser effect on the increase in the leaf area.

The results of the carbohydrate analyses of the leaves and culms and roots of the rice plants are given in the following tables (Tables XIII to XVIII). The carbohydrate contents are calculated per 100 gms. of the dry weight of the leaves and of the culms and roots. The amount of each carbohydrate present in the leaves and in the culms and roots of the unmanured and manured plants on different dates are given at the end of each fortnightly period, from 1st August to 15th October.

The results show that there is not much variation in the hexoses contents of the leaves and culms and roots of the rice plant. The quantity of hexoses present is greater in culms and roots than in the leaves. Taking the unmanured plant first the hexoses content of the leaves is higher in the early stages than that of the later stages. It is 62 mgs. in the beginning and it falls to 38 mgs. by the middle of October. In the culms and roots the hexoses content is 73 mgs. in August and falls down to 41 mgs. in October.

Cane sugar is found both in the leaves and culms and roots in the largest amounts. In the leaves of the unmanured plant it goes up from 5·4 grams (5,400 mgs.) to 8·8 grams and in the culms and roots it rises from 4·7 gms. to 9·4 gms. in October. The presence of such large quantities of cane sugar both in the leaves and culms and roots is one of the most significant facts in this investigation and it appears to be in agreement with similar observations made by Parkin [1911], Gast [1917] and Davis, Daish and Sawyer [1916] in the leaves of various plants. They regarded cane sugar as the first sugar of photosynthesis on account of its increase during the day but the presence of cane sugar in equally large amounts in culms and roots considerably weakens their conclusions. Very probably cane sugar forms the reserve material in the case of the rice plant.

Though the leaves of the rice plant are starch forming, the quantity of starch present is very little in comparison to the quantity of cane sugar. Very probably this is the characteristic of monocotyledons. The starch content of culms and roots of the unmanured plant goes up from 0·4016 gram to 1·781 gms. in October with a depression in September, when it falls down to nearly 0·3885 gm. In the leaves the starch content fluctuates between 57 mgs. and 233 mgs. and falls to 124 mgs. on the 15th October.

TABLE XIII.

Carbohydrates in gms. per 100 gms. of dry weight, 1st August.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
	Unmanured . . .	4·484		0·0571	3·028		0·4016

TABLE XIV.

Carbohydrates in gms. per 100 gms. of dry weight, 15th August.

	Unmanured . . .	0·0627	5·4723	0·0789	0·0735	4·7264	0·7500
1st August .	KNO_3	0·0611	5·4829	0·1020	0·0716	4·8933	0·7884
1st ,, .	$(NH_4)_2SO_4$ . . .	0·0690	5·9530	0·1464	0·0808	5·0661	0·9343
1st ,, .	$KNO_3+(NH_4)_2SO_4$.	0·0676	6·0744	0·2085	0·0934	5·1896	0·9918

TABLE XV.

Carbohydrates in gms. per 100 gms. of dry weight, 1st September.

	Unmanured . . .	0·0553	6·1308	0·3580	0·0740	4·8640	0·8704
1st August .	KNO_3	0·0575	6·3785	0·3713	0·0754	4·9016	0·9122
15th ,, .	,, ...	0·0559	6·4931	0·4004	0·0761	5·1009	0·9464
1st August .	$(NH_4)_2SO_4$ . . .	0·0578	6·5702	0·3898	0·0788	5·30 2	0·9662
15th ,, .	,, . . .	0·0653	6·5967	0·4247	0·0788	5·3652	1·0503
1st August .	$KNO_3+(NH_4)_2SO_4$.	0·0675	6·8095	0·5217	0·0914	5·4136	1·0605
15th ,, .	,, .	0·0661	7·1719	0·5288	0·0877	5·9693	1·0523

TABLE XVI.

Carbohydrates in gms. per 100 gms. of dry weight, 15th September.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
	Unmanured . . .	0·0403	6·8607	0·1873	0·0440	5·0530	0·3885
1st August .	KNO_3	0·0440	7·2320	0·3055	0·0467	5·4653	0·4563
15th ,, .	,, ...	0·0438	7·8282	0·3142	0·0443	5·4767	0·5369
1st September	,, ...	0·0417	7·3183	0·2451	0·0435	5·5365	0·4492
1st August .	$(NH_4)_2SO_4$ . . .	0·0458	8·3032	0·2910	0·0451	5·9809	0·5104
15th ,, .	,, ...	0·0461	8·4009	0·3466	0·0469	6·0701	0·5322
1st September	,, ...	0·0425	8·2215	0·2607	0·0492	6·1228	0·4771
1st August .	$KNO_3+(NH_4)_2SO_4$.	0·0476	8·8804	0·3443	0·0469	5·9481	0·5896
15th ,, .	,, .	0·0479	8·9381	0·3876	0·0504	6·1366	0·6457
1st September	,, .	0·0418	8·7462	0·3001	0·0493	6·1097	0·5410

TABLE XVII.

Carbohydrates in gms. per 100 gms. of dry weight, 1st October.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
	Unmanured . . .	0·0376	7·2854	0·2330	0·0320	5·4440	0·3888
1st August .	KNO_3	0·0430	7·9230	0·3285	0·0333	5·8557	0·4884
15th ,, .	,, ...	0·0394	7·9536	0·3307	0·0339	5·9171	0·5020
1st September	,, ...	0·0395	7·8535	0·3049	0·0337	5·6013	0·4405
15th ,,	,, ...	0·0387	7·6013	0·2505	0·0323	5·6267	0·4316
1st August .	$(NH_4)_2SO_4$ . . .	0·0401	8·7199	0·3717	0·0356	6·2395	0·5505
15th ,, .	,, ...	0·0407	8·7053	0·3766	0·0357	6·2773	0·5847
1st September	,, ...	0·0409	8·4681	0·3588	0·0339	6·0201	0·4925
15th ,,	,, ...	0·0404	8·2196	0·3389	0·0341	5·6709	0·4441
1st August .	$KNO_3+(NH_4)_2SO_4$.	0·0482	9·7088	0·3986	0·0462	6·7278	0·9289
15th ,, .	,, .	0·0473	9·7117	0·4426	0·0448	6·6662	0·8773
1st September	,, .	0·0406	9·5564	0·3662	0·0429	6·4281	0·7314
15th ,,	,, .	0·0436	9·4624	0·3712	0·0490	6·4791	0·6144

TABLE XVIII.

Carbohydrates in gms. per 100 gms. of dry weight, 15th October.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
	Unmanured . . .	0·0381	8·8289	0·1247	0·0413	9·4317	1·781
1st August .	KNO_3	0·0393	9·2457	0·1611	0·0461	9·9840	2·528
15th „ .	„	0·0400	9·3160	0·2009	0·0481	10·0314	2·624
1st September.	„	0·0401	9·2469	0·1730	0·0437	9·9283	2·494
15th „	„	0·0398	9·1092	0·1340	0·0432	9·9058	2·503
1st October .	„	0·0398	8·9932	0·1438	0·0419	9·6121	1·826
1st August .	$(NH_4)_2SO_4$ . . .	0·0402	9·3856	0·2124	0·0487	10·2013	2·650
15th „ .	„ . . .	0·0409	9·4231	0·2384	0·0496	10·2804	2·664
1st September.	„ . . .	0·0404	9·3896	0·1872	0·0459	10·2341	2·550
15th „	„ . . .	0·0401	9·2579	0·1703	0·0459	10·1241	2·475
1st October .	„ . . .	0·0397	9·0933	0·1412	0·0408	9·6292	1·854
1st August .	$KNO_3+(NH_4)_2SO_4$.	0·0444	10·1456	0·2561	0·0531	10·6769	3·239
15th „ .	„ .	0·0457	10·1643	0·3080	0·0568	10·7532	3·387
1st September.	„ .	0·0432	10·0268	0·2313	0·0532	10·6468	2·952
15th „	„ .	0·0423	9·6787	0·2125	0·0503	10·3997	2·740
1st October .	„ .	0·0400	9·1200	0·1443	0·0434	9·7536	1·936

The effect of manuring the rice plants in the manner described above on their photosynthetic activity can be seen from the similar carbohydrate analyses carried out of the manured plants. In general, the effect of manuring with potassium nitrate, ammonium sulphate and the mixture of the two on the photosynthetic activity of the rice plant is of the same type, as was found on its relative growth rate. A glance at the results will show that at all stages of growth from the 1st August to the 15th October the carbohydrate contents of the leaves, culms and roots of the plants manured with the mixture are higher than the carbohydrate contents of the same organs of the rice plants manured with either potassium nitrate or ammonium sulphate. At the same time the plants manured with any one of the fertilizers on

the 15th August have a greater carbohydrate content than the plants manured with the same fertilizers on other dates.

In order to make the above statement clear, fresh weights, dry weights, carbohydrate contents of the leaves, culms and roots and total carbohydrates per whole plant of the plants manured in three ways on the different dates are given in Tables XIX to XXXIV. In the unmanured plant the total carbohydrates per 100 gms. of dry weight of the whole plant rise from 3·9853 gms. to 10·1228 gms. (Table XIX). In plants manured with potassium nitrate on the 15th August, the total carbohydrates on the 15th October are 11·1304 gms. (Table XXIII). In plants manured with ammonium sulphate they are 11·3482 gms. (Table XXIV). In plants manured with the mixture of potassium nitrate and ammonium sulphate they are 12·3575 gms. (Table XXV). These are the highest values of carbohydrate contents obtained in the plants which were manured with the three fertilizers separately on the 15th August. The carbohydrate contents are lower in plants manured with any of the three fertilizers on the other dates.

In the last column of these tables the quantities of total carbohydrates present in one plant of each of the series (manured or unmanured) are given. This shows the differences in the actual quantities of carbohydrates formed in the plants treated differently on different dates.

On studying these figures for the total carbohydrates in the plants at successive stages of growth from the 1st August to 15th October, it will be seen that on two occasions the carbohydrate contents of the plants show maximum rise. In the unmanured plant the first rise in the carbohydrate contents is from 1st August to the 15th August when the carbohydrate contents rise from 3·9853 gms. to 5·5819 gms. (Table XIX). The second big rise in the carbohydrate contents is from 1st October to 15th October when the carbohydrate contents rise from 6·7104 gms. to 10·1228 gms. (Table XIX). Now in plants manured with potassium nitrate there is no greater rise in the carbohydrate contents in the 1st period as compared with the unmanured series, but in the last period the rise is from 7·3883 gms. to 11·1304 gms. (Table XXIII). In the ammonium sulphate series the 1st rise in the carbohydrates is from 3·9853 gms. to 6·1249 gms. (Table XXI) and in the mixture series it is from 3·9853 to 6·3127 gms. (Table XXII). The rise in the last period in the two latter series is the same, but the actual values of carbohydrates differ on account of the greater increase in the carbohydrate contents of the plants of the mixture series in the intervening period from 15th August to 1st October.

When the plants are manured on 15th August the 1st rise from 1st to 15th August is the same in all cases, but in the second period from 15th August to 1st September in the potassium nitrate series and ammonium sulphate series there is an increase of nearly one gram, while in the plants of the mixture series there

is an increase of nearly two grams. In the second big rise in the carbohydrate contents in the last stage, the increase is nearly the same in the plants otall the manured series. When the plants are manured on the 1st or 15th September or 1st October, there is a greater rise in the carbohydrate contents in the plants of the mixture series than in the plants of the potassium nitrate series or ammonium sulphate series in subsequent periods.

TABLE XIX.

Fresh weights and dry weights and total carbohydrates.

Unmanured plants.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry wt. of the whole plant	Total carbohydrates present per plant
1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	297·400	48·9484	7·5566	5·8648	6·7104	3·1494
15th „ .	346·500	76·6260	8·9916	11·2540	10·1228	8·1047

TABLE XX.

Plants manured with potassium nitrate on 1st August.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	25·390	2·7720	5·6460	5·7534	5·6997	0·1581
1st September .	75·420	8·5460	6·8073	5·8892	6·3482	0·5327
15th „ .	205·200	22·4868	7·5815	5·9683	6·7749	1·5186
1st October .	365·500	62·2120	8·2945	6·3774	7·3359	4·3321
15th „ .	430·000	92·9260	9·4461	12·5581	11·0021	10·7885

TABLE XXI.

Plants manured with ammonium sulphate on 1st August.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry wt. of the whole plant	Total carbohydrates present per plant
1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	29·200	3·1365	6·1685	6·0813	6·1249	0·1919
1st September .	78·920	9·4483	7·0178	6·3472	6·6825	0·6260
15th „ .	229·500	27·7440	8·6400	6·5364	7·5882	2·0308
1st October .	380·430	65·7188	9·1317	6·8255	7·9786	4·9834
15th „ .	445·100	95·5660	9·6384	12·9000	11·2692	11·4011

TABLE XXII.

Plants manured with potassium nitrate and ammonium sulphate on 1st August.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	32·740	3·5940	6·3506	6·2748	6·3127	0·2269
1st September .	88·520	10·4964	7·3987	6·5655	6·9821	0·7259
15th „ .	255·100	29·0202	9·2723	6·7616	8·0169	2·2431
1st October .	461·800	74·0128	10·2016	7·5883	8·8949	6·2730
15th „ .	513·100	110·3620	10·4461	13·9690	12·2075	14·2394

TABLE XXIII.

Plants manured with potassium nitrate on 15th August.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	78·190	9·6596	6·9494	6·1234	6·5364	0·6199
15th „ .	216·100	26·4734	8·1862	6·0579	7·1220	1·8225
1st October .	379·800	65·1660	8·3237	6·4530	7·3883	4·6044
15th „ .	450·100	95·6360	9·5569	12·7040	11·1304	11·3123

TABLE XXIV.

Plants manured with ammonium sulphate on 15th August.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry wt. of the whole plant	Total carbohydrates present per plant
1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	86·930	10·5995	7·0867	6·4943	6·7905	0·7140
15th „ .	235·300	28·4643	8·7936	6·6492	7·7214	2·1383
1st October .	403·510	69·0900	9·1226	6·8977	8·0101	5·2658
15th „ .	454·800	97·8600	9·7024	12·9940	11·3482	11·7758

TABLE XXV.

Plants manured with potassium nitrate and ammonium sulphate on 15th August.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	105·940	13·0232	7·7668	7·1093	7·4380	0·9615
15th „ .	278·420	33·4556	9·3736	6·8327	8·1031	2·6313
1st October .	483·400	74·6992	10·1556	7·7029	8·9292	6·3719
15th „ .	532·600	113·9180	10·5180	14·1970	12·3575	14·9338

TABLE XXVI.

Plants manured with potassium nitrate on 1st September.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ .	218·700	26·4064	7·6051	6·0292	6·8171	1·7539
1st October .	386·820	65·5400	8·1979	6·0755	7·1367	4·4390
15th „ .	414·600	88·4820	9·4600	12·4660	10·9630	10·2396

TABLE XXVII.

Plants manured with ammonium sulphate on 1st September.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry. wt. of the whole plant	Total carbo hydrates present per plant
1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5000	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	224·950	27·3517	8·5247	6·4721	7·4984	1·9891
1st October .	390·020	65·9426	8·8678	6·5465	7·7071	4·8199
15th „ . .	427·700	91·9760	9·6172	12·8300	11·2236	10·8213

TABLE XXVIII.

Plants manured with potassium nitrate and ammonium sulphate on 1st September.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	259·300	30·7656	9·0881	6·7000	7·8940	2·3594
1st October .	442·230	72·3592	9·9632	7·2024	8·5828	5·9306
15th „ . .	491·000	105·7880	10·3013	13·6520	11·9766	13·3454

TABLE XXIX.

Plants manured with potassium nitrate on 15th September.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	338·700	65·5744	7·9449	6·0906	7·0177	4·3775
15th „ . .	404·600	86·8820	9·2830	12·4520	10·8660	9·8950

TABLE XXX.

Plants manured with ammonium sulphate on 15th September.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry wt. of the whole plant	Total carbohydrates present per plant
1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	402·600	68·2904	8·5989	6·1491	7·3737	4·7345
15th „ . .	421·200	89·9280	9·4683	12·6450	11·0566	10·5248

TABLE XXXI.

Plants manured with potassium nitrate and ammonium sulphate on 15th September.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	453·700	73·3772	9·8772	7·1364	8·5068	5·9235
15th „ . .	483·500	104·5360	9·9335	13·1900	11·5615	12·7413

TABLE XXXII.

Plants manured with potassium nitrate on 1st October.

1st August .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ . .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October	297·400	48·9480	7·5560	5·8648	6·7104	3·1494
15th „ . .	360·700	78·9640	9·1767	11·4800	10·3284	8·5099

TABLE XXXIII.

Plants manured with ammonium sulphate on 1st October.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates per 100 gms. of dry wt. of the whole plant	Total carbohydrates present per plant
1st August. .	5·219	0·6753	4·5411	3·4296	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	297·400	48·9480	7·5560	5·8648	6·7104	3·1494
15th „ .	385·400	82·6660	9·2742	11·5240	10·3991	8·9738

TABLE XXXIV.

Plants manured with potassium nitrate and ammonium sulphate on 1st October.

1st August. .	5·219	0·6753	4·5411	3·4294	3·9853	0·0257
15th „ . .	19·315	1·7329	5·6139	5·5500	5·5819	0·0968
1st September .	62·850	7·0040	6·5440	5·8084	6·1764	0·4290
15th „ .	180·470	19·6400	7·0883	5·4855	6·2869	1·1995
1st October .	297·400	48·9480	7·5560	5·8648	6·7104	3·1494
15th „ .	401·400	86·0350	9·3043	11·7330	10·5186	9·4732

It appears that there are two periods of maximum photosynthetic activity in the rice plants. The first period of maximum photosynthetic activity is between

1st August and 15th August and the second period of maximum photosynthetic activity is from 1st October to 15th October. The first period of maximum photosynthetic activity coincides with the period when the largest amount of the leaf surface is produced as shown in the leaf area ratios given above (Tables X, XI, XII), the second period of photosynthetic activity coincides with the flowering period. At that time there is no second increase in the leaf area as can be seen from the leaf area ratio. The cause for the second maximum period of photosynthetic activity is perhaps to be sought for in the external conditions such as sun-light and temperature.

If the second period of maximum photosynthetic activity is at the time of flowering, *i.e.*, between the 1st October and the 15th October, what is the effect on the assimilatory activity, of manuring the plants with each of the three fertilizers on the 1st October? The results show that it has nearly no effect as in the potassium nitrate series, the ammonium sulphate series and the mixture series, the rise in the carbohydrate contents is 3·6 gms., 3·7 gms. and 3·8 gms. respectively, while in the unmanured plant the rise is 3·4 grams (Tables XIX, XXXII, XXXIII, XXXIV). It appears to be a waste of fertilizer to manure the plants so late as the flowering period or later as is proposed by several investigators. If the plants need to be manured it should be done early before the 15th August.

The above results of the relative growth rate of the rice plant and of their carbohydrate contents indicate the value of the mixture of ammoniacal nitrogen and nitrate nitrogen as a fertilizer, as compared to the value of nitrate nitrogen or ammoniacal nitrogen used singly on equal nitrogen basis. It might be argued that the greater beneficial effect on the growth and yield of grain of the rice plant manured with a mixture of potassium nitrate and ammonium sulphate is not due to the mixture of two forms of nitrogen supplied, but is caused by the presence of potash and nitrogen together in the mixture, while the absence of potash for the plants manured with ammonium sulphate alone and the low value of the nitrate nitrogen for the plants manured with potassium nitrate bring about a decreased growth and yield of the rice plant, as compared to the growth and yield of the rice plant manured with the mixture.

This point therefore remained to be cleared and it was undertaken in the next season. The rice plants were grown in four separate beds and on the 15th August one bed was manured with the nitrate of soda instead of potassium nitrate. Second bed was manured with ammonium sulphate and a third bed was manured with a mixture of sodium nitrate and ammonium sulphate. The fourth bed was not manured with nitrogen in any form. It was made certain that the soils did not lack in phosphates by putting a sufficient doze of super-phosphates in all the

beds. The size of each bed was 90 sq. ft. The total dry weight of the plants and those of the straw and the grain are given in the following table.

TABLE XXXV.

Nature of manure	Weight of straw fn lbs.		Weight of grain in lbs.		Total weight of the plant		Ratio of straw wt. to grain wt.
	lbs.	oz.	lbs.	oz.	lbs.	oz.	
Unmanured . .	10	5	3	11	14	0	3·3 : 1
$NaNO_3$ . . .	13	14	5	8½	19	6½	2·8 : 1
$(NH_4)_2SO_4$ . .	15	13	6	2	21	15	2·6 : 1
$NaNO_3+(NH_4)_2SO_4$.	20	6	7	6	27	12	2·9 : 1

The results obtained here are similar to those given in Table VI. In Table VI the yield of grain in plants manured with the mixture of potassium nitrate and ammonium sulphate is double the yield obtained with unmanured plants. The yield of grain obtained above from the plants manured with a mixture of sodium nitrate and ammonium sulphate is double the yield of grain obtained with the unmanured plants. The above results conclusively show that the increased yield of grain and the highest growth rate obtained in plants manured with the mixture are due to the use of mixed forms of ammoniacal and nitrate nitrogen and not due to the presence of both potash and ammoniacal nitrogen as was argued above, as the results given in Table XXXV show, where potash is omitted altogether.

Another important feature of the results given in Table XXXV is the greater increase in the dry weight of the straw as compared to the yield of the grain. In Table VI the ratio of weight of straw to the weight of grain is 2 : 1 in the plants manured with the mixture of potassium nitrate and ammonium sulphate, while the ratio of the straw to grain in Table XXXV for the plants manured with mixture is 2·9 :1, thus indicating that the increase in weight of the straw is greater than in the previous experiments. This holds good for the plants in sodium nitrate and ammonium sulphate series. In the unmanured plants the ratio is 3·3:1. This increase in straw weight is not due to the use of sodium nitrate instead of potassium nitrate as a similar increase in straw weight is found in the unmanured plants. So it is possible that the increase in the straw weight is caused by the addition of superphosphates in all the four beds before transplantation to remove the deficiency of superphosphates in the soil and the plants in the three beds were then manured with three fertilizers on the 15th August and the fourth one was kept unmanured. Whether the increase in straw without increase in yield of grain is caused by the addition of superphosphates or not can be seen from the investigation described below.

As the results obtained above have shown that the best growth is made by the plants when manured with any form of nitrogen in the middle of August or before and the mixture of nitrate nitrogen and ammoniacal nitrogen gives better growth and yield than any one form of nitrogen used singly by itself, it was undertaken to study similarly the effects of applications of superphosphates on the growth and yield of the rice plant. It would be of interest to determine whether the beneficial effect of early manuring of plants with nitrogen holds good only for nitrogen and is specific in that case only, or if it holds good for other manures also.

It is possible that when the growth activities are at their highest pitch, the manuring of the plants with fertilizers other than those containing nitrogen will also result in enhanced growth and reproduction as compared to the growth and reproduction resulting from the application of the manure later than 15th August. If this assumption is found to be correct, it would be an important result of this investigation, as it will definitely establish an important fact of manuring the rice plant once for all.

The rice plants were transplanted in separate beds just as in the previous year and the beds were manured with a mixture of sodium nitrate and ammonium sulphate before transplantation.

One batch of manured plants was then given a doze of superphosphates on the 1st August, a second batch was given a doze of superphosphates on the 15th August, a 3rd batch on the 1st September, a fourth on the 15th September and the fifth on 1st October. The sixth batch of plants was kept totally unmanured with the mixture of sodium nitrate and ammonium sulphate and with superphosphates also. This batch was kept totally unmanured to test the results obtained before with the unmanured plants in the previous year's experiments. The fresh weights, dry weights of leaves and culms and roots, volume of roots, total leaf area, water content of the plants of each batch were determined every fifteen days after manuring was done in the previous year. The same was done for the unmanured batch of plants from the 1st August to 15th October. On the 15th November the plants were harvested and total dry weight of the plants and the respective weights of the straw and grain were determined in all the series.

The following tables (Tables XXXVI—XLI) give the average fresh weight of the leaves of one whole plant, dry weight of one whole plant, volume of roots and total leaf area per plant of the manured and unmanured plants at different stages of growth. In the tables below, the unmanured means that the plants are unmanured with both, the mixture of two forms of nitrogen and superphosphates. As the beds were manured with sodium nitrate and ammonium sulphate prior to transplantation, it is expected that the relative growth rate will reach its maximum point earlier than it was the case in the previous series of experiments. It was

so found to be the case in previous series when the plants were manured with the mixture on the 1st August.

Taking first the batch of plants wholly unmanured with the mixture and superphosphates, the fresh weight of the plants increases from 6·286 gms. on the 1st August to 365·7 gms. on the 15th October. The total leaf area on the 15th October is 5574 and the dry weight on the same date is 79·500 gms. on 15th October and 106 gms. on the 15th November (Tables XXXVI, XLI and XLII). On comparing these results with those given for the unmanured plants in Tables I—VI, it will be seen that the yield of straw and grain are nearly the same in the second year's experiments. The ratio of weight of straw to the weight of grain is 2·8:1 as in the former case (Table XLII.)

In plants manured with the mixture before transplantation and superphosphates on 1st August the fresh weight increases to 592·6 gms. and total leaf area to 10420 sq. cm. on the 15th October. The dry weight per plant increases to 184·3 gms. on the 15th November (Tables XLI and XLII). On comparing these values with those obtained for the plants manured with superphosphates on the 15th August it will be seen that there is no difference between them. In both cases the final weight of plants as well as the weights of straw and grain are equal. But when the plants are manured with superphosphates later than 15th August, it results in decreasing yield of straw and grain as could be seen from the results obtained in the case of plants manured on the 1st or 15th September or the 1st October. It appears from the results that manuring the plants with superphosphates on the 15th September or 1st October has no effect on the growth of the plant as the final dry weights, leaf areas and the fresh weight of the plants in two cases are nearly equal. It will also be seen that the ratio of the weight of the straw to the weight of grain is 2:1 in the last three cases of manuring and this is the ratio obtained in the manured plants with the mixture of nitrate and ammonium sulphate in the previous year's experiments (Table VI).

The results here show conclusively that, as in the case of nitrogen fertilizers the plants should be manured early in August in order to get the maximum effect. Whatever manures are supplied later in the season it will always result in decreasing gain.

Another important feature in these results obtained with the manuring of plants with superphosphates is the greater effect of this manure on the vegetative growth than on the reproduction. It will be seen by comparing the results obtained in this investigation that there is a greater increase in yield of the straw than in the yield of grain. If the results obtained on the 1st August are compared with those obtained on the 15th September (Table XLII) it will be seen that there is a proportionately greater yield of straw than of the grain on account of manuring

with superphosphates on the 1st August. There is a gain of 32 gms. in the straw weight and only a gain of 8 gms. in the weight of grain. Similar conclusions have already been arrived at as a result of experiments to test again the greater value of a mixture of nitrate nitrogen and ammoniacal nitrogen as a fertilizer than any one form used singly. The results were given in Table XXXV. Here also the greater increase in straw weight as compared to the increase in the yield of grain is due to the manuring of the beds with superphosphates before transplantation.

TABLE XXXVI.

Fresh and dry weights, leaf area and volume of roots of wholly unmanured plant and of plants manured with superphosphates.

Date of manuring	Nature of manure	Fresh wt. of the leaves per plant in gms.	Total fresh wt. per plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cms.	Total water content in gms.	Dry wt. in gms.
		1st August 1931.					
	Wholly unmanured .	1·32	6·286	0·75	142·1	5·590	0·6958

TABLE XXXVII.

15th August.

	Wholly unmanured .	12·50	50·32	7·0	1058·4	43·91	6·41
1st Aug. . .	Superphosphates .	20·50	82·50	8·7	1654·8	72·49	10·00

TABLE XXXVIII.

1st September.

	Wholly unmanured .	39·88	171·1	19·5	3422·4	149·9	21·20
1st Aug. . .	Superphosphates .	66·45	294·6	29·7	5568·4	255·2	38·40
15th „ . .	„ .	63·20	277·2	28·0	5288·8	240·3	36·84

TABLE XXXIX.

15th September.

	Wholly unmanured .	71·42	297·3	36·5	4842·0	247·60	49·72
1st Aug. . .	Superphosphates .	107·84	456·6	48·5	7848·0	383·40	73·17
15th „ . .	„ .	109·82	465·1	51·7	8004·0	390·97	74·15
1st Sep. . .	„ .	98·52	427·0	45·7	7012·0	358·80	68·54

TABLE XL.

Fresh and dry weights, leaf area and volume of roots of wholly unmanured plant and of plants manured with superphosphates.

Date of manuring	Nature of manure	Fresh wt. of the leaves per plants in gms.	Total fresh wt. per plant in gms.	Volume of roots in c.c.	Total leaf area in sq. cm.	Total water content in gms.	Dry wt. in gms.
		1st October.					
	Wholly unmanured .	75·1	331·7	58·40	5284·0	269·9	61·78
1st August .	Superphosphates .	126·2	547·9	73·60	9008·0	449·5	98·34
15th „ . .	„ .	127·2	555·2	74·00	9062·0	455·8	99·36
1st September .	„ .	118·2	520·2	72·00	8460·0	425·7	94·50
15th „ . .	„ .	110·4	499·2	69·75	8202·0	407·3	91·81

TABLE XLI.

15th October.

	Wholly unmanured .	78·90	365·7	60·5	5574·0	286·19	79·50
1st August .	Superphosphates .	138·60	592·6	76·5	10420·0	462·89	128·74
15th „ . .	„ .	139·30	595·6	76·5	10482·0	465·59	130·01
1st September .	„ .	129·31	545·7	74·0	9389·0	426·85	118·87
15th „ . .	„ .	120·30	502·0	72·5	8664·0	392·94	109·08
1st October .	„ .	119·40	500·2	72·2	8600·0	391·50	108·70

TABLE XLII.

The weight of straw, grain and total dry weight.

Date of manuring	Nature of manure	Weight of straw per plant in gms.	Weight of grain per plant in gms.	Total dry weight per plant in gms.	Ratio of straw to grain
	15th November.				
	Wholly unmanured . . .	78·2	27·8	106·0	2·8 : 1
1st August .	Superphosphates . . .	128·0	56·3	184·3	2·2 : 1
15th „ . .	„ . . .	127·5	56·5	184·0	2 : 1
1st September .	„ . . .	105·5	52·1	157·6	2 : 1
15th „ . .	„ . . .	96·0	48·7	144·7	2 : 1
1st Oct. . .	„ . . .	96·5	48·8	145·3	2 : 1

The relative growth rates of the plants manured with superphosphates at different stages of growth were also calculated as was done in previous year and the following table (Table XLIII) gives the values of the relative growth rates calculated according to the formula given above.

The carbohydrate analyses of the leaves, culms and roots of the wholly unmanured plants and of plants manured with superphosphates at different stages of growth were made every fortnight as was done in the previous year to see the effect of superphosphates on the carbohydrate contents of the plants. The results of these carbodydrate analyses showing the hexoses, cane sugar and starch contents of leaves, culms and roots are given in Tables XLIV—LV. The results are given as percentages of dry weight of the respective plant parts.

TABLE XLIII.

Relative growth rates of the rice plants manured with superphosphates at different stages of growth.

Date of manuring	1st Aug. to 15th Aug.	15th Aug. to 1st Sep.	1st Sep. to 15th Sep.	15th Sep. to 1st Oct.	1st Oct. to 15th Oct.	15th Oct. to 15th Nov.
1st August .	*2·66*	1·34	0·64	0·29	0·27	0·17
15th „ .	2·22	*1·74*	0·70	0·29	0·26	0·17
1st September .	2·22	1·19	*1·17*	0·32	0·22	0·14
15th „ .	2·22	1·19	0·81	*0·61*	0·17	0·14
1st October .	2·22	1·19	0·81	0·24	*0·56*	0·14

TABLE XLIV.

Carbohydrates in gms. per 100 gms. of dry weight.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
			1st August.				
	Wholly unmanured .		4·713 0	0·0580	3·8770		0·4708

TABLE XLV.

15th August.

	Wholly unmanured .	·0·0597	4·9742	0·2798	0·0745	4·2840	0·7195
1st August .	Superphosphates .	0·0657	6·8342	0·5047	0·0910	5·5760	0·9007

TABLE XLVI.

1st September.

	Wholly unmanured .	0·0561	6·0489	0·3896	0·0609	4·8210	1·2270
1st August .	Superphosphates .	0·0637	7·7143	0·6792	0·7300	5·8540	1·4190
15th ,, .	,, .	0·0610	7·4779	0·6423	0·7310	5·7488	1·4190

TABLE XLVII.

15th September.

	Wholly unmanured .	0·0453	6·7626	0·4312	0·0442	5·2747	1·3210
1st August .	Superphosphates .	0·0501	9·0379	0·7496	0·0486	6·3633	1·5230
15th ,, .	,, .	0·0501	9·1039	0·7361	0·0492	6·3958	1·5380
1st September .	,, .	0·0458	8·5761	0·7174	0·0477	6·1703	1·5220

TABLE XLVIII.

Carbohydrates in gms. pr. 100 gms. of dry weight.

Date of manuring	Nature of manure	Leaves			Culms and roots		
		Hexoses	Cane sugar as hexoses	Starch as hexoses	Hexoses	Cane sugar as hexoses	Starch as hexoses
		1st October.					
	Wholly unmanured .	0·0418	8·2801	0·4596	0·0396	5·3584	1·3370
1st August .	Superphosphates .	0·0462	9·9147	0·7775	0·0425	7·2984	1·5640
15th „ .	„ .	0·0462	9·9257	0·8129	0·0431	7·2969	1·5650
1st September .	„ .	0·0461	9·7398	0·7019	0·0414	7·0736	1·4750
15th „ .	„ .	0·0439	9·4620	0·6144	0·0407	7·0122	1·4580

TABLE XLIX.

15th October.

	Wholly unmanured .	0·0349	8·8691	0·4400	0·0445	9·4945	2·2530
1st August .	Superphosphates .	0·0393	10·8307	0·5992	0·0567	11·5133	4·9640
15th „ .	„ .	0·0395	10·8405	0·5862	0·0574	11·5125	4·9830
1st September .	„ .	0·0387	10·0313	0·5716	0·0523	10·4476	4·5140
15th „ .	„ .	0·0378	9·7669	0·5514	0·0519	10·0880	4·5210
1st October .	„ .	0·0378	9·6962	0·5457	0·0518	10·0581	4·6120

Taking the wholly unmanured plant first it will be seen that the hexoses and cane sugar contents increase from 4·713 to 8·9 gms. on the 15th October in the leaves and from 3·877 to 9·539 gms. in culms and roots. These values for the hexoses and cane sugar agree with those obtained in the case of unmanured plants in the previous year (Tables XIII-XVIII). The starch contents are higher in the series than the strach contents of the previous year series.

Taking the plants manured with superphosphates on the 1st or 15th August it will be seen that there is a slight increase in the carbohydrate contents of these plants as compared to the carbohydrate contents of the plants manured with the

mixture of nitrate and ammonium sulphate on the 1st or 15th August in the previous year (Tables XXII and XXV). The larger amounts of starch present in the leaves, culms and roots of the plants manured with superphosphates in addition to the mixture is a special feature though there is no direct evidence to show that the increase in the starch contents is due to manuring with superphosphates.

Table L.

Total fresh and dry weights of the whole plant and carbohydrates in leaves, in culms and roots and in the whole plant at different stages of growth.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates in the whole plant per 100 gms. of dry wt.	Total carbohydrates per bunch in gms.
			Wholly unmanured.			
1st August .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ .	50·326	6·4123	5·3138	5·0785	5·1961	0·3325
1st September .	171·130	21·2090	6·4946	6·1090	6·3018	1·3302
15th „ .	297·320	49·7250	7·2392	6·6400	6·9396	3·4032
1st October .	331·720	61·7800	8·7816	6·7350	7·7583	3·5632
15th „ .	365·700	79·5050	9·3480	11·7920	10·5700	8·8272

Table LI.

			Plants manured with superphosphates on 1st August.			
1st August .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ .	82·500	10·0004	7·4047	6·5677	6·9860	0·5926
1st September .	294·600	38·4072	8·4572	7·3460	7·9016	2·9880
15th „ .	456·650	73·1700	9·8376	7·9350	8·8863	6·2620
1st October .	547·930	98·3440	10·7385	8·9050	9·8217	9·2256
15th „ .	592·620	129·7434	11·4692	16·5340	14·0016	19·2250

TABLE LII

Total fresh and dry weights of the whole plant and carbohydrates in leaves, in culms and roots and in the whole plant at different stages of growth.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates in the whole plant per 100 gms. of dry wt.	Total carbohydrates per bunch in gms.
Plants manured with superphosphates on 15th August.						
1st August .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ . .	50·326	6·4123	5·3138	5·0785	5·1961	0·3325
1st September .	277·170	36·8408	8·1813	7·2410	7·7111	2·8072
15th „ . .	465·120	74·1500	9·8901	7·9830	8·9365	6·4027
1st October .	555·200	99·3660	10·7849	8·9050	9·8217	9·4116
15th „ . .	595·600	130·0134	11·4662	16·5530	14·0096	19·3882

TABLE LIII

Plants manured with superphosphates on 1st September.

1st August .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ . .	50·326	4·4123	5·3138	5·0785	5·1961	0·3325
1st September .	171·130	21·2090	6·4946	6·1090	6·3013	1·3302
15th „ . .	427·420	68·5400	90·3394	7·7400	8·5392	5·6325
1st October .	520·240	94·5000	10·4879	8·5900	9·5389	8·6464
15th „ . .	545·730	118·8740	10·6416	15·0140	12·8278	16·1513

TABLE LIV

Total fresh and dry weights of the whole plant and carbohydrates in leaves, in culms and roots and in the whole plant at different stages of growth.

Date	Total fresh wt. in gms.	Total dry wt. in gms.	Total carbohydrates in leaves per 100 gms. of dry wt.	Total carbohydrates in culms and roots per 100 gms. of dry wt.	Total carbohydrates in the whole plant per 100 gms. of dry wt.	Total carbohydrates per bunch in gms.
Plants manured with superphosphates on 15th September.						
1st August. .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ . .	50·326	6·4123	5·3138	5·0785	5·1961	0·3325
1st September .	171·130	21·2090	6·4946	6·1090	6·3013	1·3302
15th „ .	297·320	49·7250	7·2392	6·6400	6·9396	3·4032
1st October .	499·200	91·8080	10·1204	8·5110	9·3157	8·2788
15th „ .	502·000	109·0810	10·3562	14·6540	12·5050	14·4342

TABLE LV

Plants manured with superphosphates on 1st October.

1st August .	6·286	0·6958	4·7710	4·3478	4·5594	0·0276
15th „ .	50·326	6·4123	5·3138	5·0785	5·1961	0·3325
1st September .	171·130	21·2090	6·4946	6·1090	6·3013	1·3302
15th „ .	297·320	49·7250	7·2392	6·6400	6·9396	3·4032
1st October .	331·720	61·7800	8·7816	6·7350	7·7583	3·5632
15th „ .	500·000	108·7000	10·2797	14·7220	12·5008	14·3379

In Tables L to LV the total carbohydrate contents of leaves, culms and roots and of the whole plant as percentage of dry weights are given. The total carbohydrates per bunch of each series are also given. The total carbohydrates per 100 gms. of dry weight of wholly unmanured plants increase from 4·55 gms. to 10·57 gms. on the 15th October. These values agree with the total carbohydrate values of the unmanured plants as determined in the previous year (Table XIX). In plants manured with superphosphates on the 1st or 15th August the total carbohydrates increase to 14 gms. on the 15th October (Tables LI and LII). So there is an

increase of two per cent. in the carbohydrate contents of the plants manured with superphosphate on the 1st or 15th August over the carbohydrate contents of the plants manured with the mixture of nitrate and ammonium sulphate on the 1st or 15th August (Tables XXII and XXV). The total carbohydrate content per plant is also very much higher in the plants of the same series than the carbohydrate contents per plant in the previous two series.

The total carbohydrate values per 100 gms. of dry weight of the whole plant or per plant in the case of manuring with superphosphates on the 1st September, 15th September or 1st October (Tables, LIII, LIV, LV) are nearly equal to carbohydrate values in the plants manured with the mixture on the 1st or 15th August in the previous year (Tables XXII and XXV).

Conclusions.

The relative growth rate of the rice plant reaches its maximum in August and then it begins to fall. The leaf area ratio, that is, the leaf area per unit dry weight of the plant also reaches its maximum early in August after which there is a continuous fall in the leaf area ratio. These results suggest that the best period of manuring the plants, so as to obtain the maximum effect would be the period, when the relative growth rate of the plant is highest. This has been found to be the case when the above assumption was put to the test by making the growth analyses of the plants manured at different stages of growth. The fresh and dry weights of the rice plants, their leaf areas and the volumes of the roots when manured on the 15th August with a nitrogenous fertilizer such as a nitrate or ammonium sulphate or both together on equal total nitrogen basis are higher than when the plants are manured at any other period.

The results obtained in this investigation have also brought to light the fact, that a mixture of ammoniacal nitrogen and nitrate nitrogen has more beneficial effects on the growth and yield of grain of the rice plant than any one form of nitrogen used singly in equal proportion. The maximum growth and yield are obtained when the plants are manured in August with a mixture of two forms of nitrogen. Even when the plants are manured with the mixture of the two forms of nitrogen, on any other stage of growth, though the growth and yield are comparatively less than those of the plants manured in August, they are higher than the growth and yield of plants manured with a single form of nitrogen on corresponding stages of growth. So at all stages of growth the mixture of two forms of nitrogen has been found to be a better fertilizer than any one form of nitrogen used singly.

The ratio of the dry weight ot the straw to the weight of the grain in unmanured plants is 2·8 : 1, in the plants manured with nitrate nitrogen it is 2·6 : 1, in plants manured with ammoniacal nitrogen it is 2·4 : 1 and in plants manured with

the mixture of the two forms of nitrogen is 2: 1 (Table VI). The results indicate that there is greater production of grain as compared to the vegetative growth when the plants are manured with the mixture of two forms of nitrogen.

The beneficial effects of manuring with the mixture of two forms of nitrogen are also shown by the carbohydrate analyses of the rice plants unmanured as well as manured with one form of nitrogen. The carbohydrate contents of plants manured with the mixture at any stage of growth are higher than the carbohydrate contents of plants manured with one form of nitrogen only on the corresponding stage of growth. The higher carbohydrate contents conclusively indicate greater vigour and greater growth of the plants than those with lesser carbohydrate contents.

The carbohydrate contents of the plants manured with the mixture of two forms of nitrogen in August are higher than the carbohydrate contents of plants manured at other stages of growth. The same is true in the case of plants manured with the nitrate nitrogen or ammoniacal nitrogen. These results again support the conclusion that early manuring results in better growth than late manuring.

The manuring of plants with nitrate nitrogen at the flowering stage results in no increase in yield of grain or straw as compared with the yield and growth of the totally unmanured plants (Table VI). The same holds good for the carbohydrate contents of the plants in the two cases. These results do not support the view that the rice plants should be manured at the flowering stage when the assimilatory activity is at its maximum. It is true, as shown by Dastur and Chinoy [1932] that the photosynthetic activity reaches its maximum at the flowering stage (*i.e.*, in the first half of October) but the manuring of plants at that stage does not in any way increase the production of carbohydrate material. It is very necessary that the various raw materials must be present in the plants in proper form and quantity, so that when the period of maximum assimilatory activity arrives, they may be rapidly synthesized into proper food substances. In order that the raw materials may be ready in proper forms suitable for rapid assimilation it is necessary that the manures should be supplied to the plants at an early stage, when the growth is most rapid. It appears from the results obtained above that an application of nitrogen in any form exactly at the time when the plant passes through the most vigorous period of assimilatory activity results in no increased output of food substances and therefore, it appears that the nitrogen supplied, even though it may be absorbed by the plant is not made use of by the plant. This is very likely as many instances are now known when the absorption and accumulation of certain salts occur in plants on account of the permeability of protoplasm even though these salts are of no use to them either for the purpose of growth or reproduction.

It is also natural that nitrogen should be supplied at a period when there is maximum expansion of vegetative parts so that it may result in an enhanced

growth. This has been found to be true as the results of the relative growth rate of the plant will show.

The beneficial effect of early manuring on the growth and yield of the rice plants not only holds good in the case of nitrogen only, but holds good for other manures also as could be seen from the results obtained with superphosphate (Tables XXXVI—XLII). A greater increase in dry weight of the plant as well as in the yield is obtained when the rice plants are manured with superphosphates in August than when the manuring is done later in September or October. The results of carbohydrate analyses of the plants manured with superphosphates also point to the same conclusions (Tables XLIII—LV). The carbohydrate contents of the rice plants manured with superphosphates on the 15th September and the 1st October show no great increase (Tables LIV and LV) as compared with the carbohydrate contents of the rice plants manured with the mixture of two forms of nitrogen (Tables XXXI and XXXIV). (Comparison with plants manured with the mixture of two forms of nitrogen is here made as the plants manured with superphosphates were also manured with the mixture of two forms of nitrogen at the time of transplantation to avoid deficiency of nitrogen.)

Manuring with superphosphates results in a proportionately greater vegetative growth than reproductive growth as could be seen from the ratios of the dry weight of the straw to the yield of grain. There is proportionately greater increase in the straw weight.

In plants manured with superphosphates on the 15th September and 1st October, the ratios of the straw weight to grain weight are 2 : 1 in both cases. Now this is the ratio for the plants manured with the mixture of two forms of nitrogen. As these plants were manured with the mixture of two forms of nitrogen at the time of transplantation, the ratio 2 : 1 shows that there is no greater increase in the straw weight as compared to the increase in grain weight. The actual straw weight and grain weight per plant in the plants manured with superphosphates on those two dates are 96 and 48 grms. respectively (Table XLII). The straw weight and grain weight of the plants manured with the mixture of two forms of nitrogen on the 15th August in Table VI are 100 grms. and 48 grms. respectively. So the results obtained with additional superphosphate manuring show no increase in straw weight and grain weight. The results again point out that if manuring with the mixture of two forms of nitrogen is done at the time of transplantation (20th July) *i.e.*, earlier than 1st August, beneficial effects are still obtained.

The conclusion that superphosphates increase the vegetative growth in greater proportion than the reproductive growth is supported by the results given in Table XXXV when the plants in four beds were first manured with superphosphates before transplantation. The addition of superphosphates has increased the ratio of

straw weight to grain weight of plants unmanured with any form of nitrogen 2·8 : 1 to 3·3 : 1. Similarly the ratios of straw weight to grain weight are higher in plants manured with nitrogen also, than they are in plants unmanured with superphosphates (Table VI) and manured with nitrogen.

The results discussed above are obtained with plants grown in very small plots in the garden of the Institute. It is essential that these results be tested in the fields. This is being attempted now at several places in the Bombay Presidency.

Summary.

It was undertaken (*a*) to determine the relative growth rate of the rice plant, (*b*) to determine the best period of manuring the rice plant so as to obtain the maximum effect with a minimum expenditure of fertilizer, (*c*) to study the effect of nitrate nitrogen, ammoniacal nitrogen and a mixture of the two forms of nitrogen on equal total nitrogen basis on the growth and yield of the rice plant, and (*d*) to make carbohydrate analyses of the rice plants to determine the effect on the carbohydrate contents of the plants manured in any one of these ways at different stages of growth.

2. Rice seedlings of the Columba variety No. 42 were transplanted in July 1930 in specially prepared beds. One batch of plants was manured with potassium nitrate, another with ammonium sulphate and a third with a mixture of potassium nitrate and ammonium sulphate on the 1st August. Similarly three other unmanured batches were manured with three forms of manuring on the 15th August. The same was done for another three batches on the 1st September and the process followed with other unmanured batches of plants on the 15th September and 1st October respectively. Each batch was thus manured with one type of manure once only. A batch of plants was kept unmanured.

3. The fresh and dry weights of leaves, of the culms and roots, volume of the roots and leaf area were determined every fortnight for all the batches manured in three different ways at the end of the five stages of growth. Similar records were kept for the unmanured plants.

4. The relative growth rates of the unmanured and manured plants were determined according to the formula R. G. R.=$\text{Log}_e W_1 - \text{Log}_e W_0$, where W_0 and W_1 are the dry weights of the whole plant on two consecutive periods. Similarly the relative growth rates of the leaves were also separately determined. The leaf area ratio, *i.e.*, the total leaf area per unit dry weight was also determined in all the batches.

5. The carbohydrate analyses of the leaves, culms and roots of the plants of all batches were made according to the methods, recently published by Dastur and Samant [1933].

6. The relative growth rate of the rice plant reaches its maximum in August. The same is true of the relative growth rate of the leaves and the leaf area ratio. This holds good for the plants of all the different batches differently treated. (Figs. 1 to 13.)

In the case of manured plants with one of the three forms of nitrogen it is noticed that the maximum relative growth rate is still reached in August. Among the three forms of manuring the mixture of nitrate and ammoniacal nitrogen is the best as the growth rate of the plants at any stage of manuring is higher than the growth rate of the plants manured with either nitrate or ammoniacal nitrogen alone (Figs. 1 to 5). The dry weights of the straw and weights of the grain of the plants follow the same rule, the maximum yield of both being obtained in plants manured with the mixture of two forms of nitrogen on the 15th August. The ratio of the straw weight to grain weight in unmanured plants is 2·8 : 1, in potassium nitrate series 2·6 : 1, in ammonium sulphate series 2·4 : 1 and the mixture series 2 : 1.

The increase in growth rate and the yield of grain of the rice plants manured on 1st October (*i.e.*, flowering stage) are negligible as compared with those of unmanured plants. In order to obtain the maximum effect with a single doze of manure the plants should be manured in August with a mixture of two forms of nitrogen.

7. The results of carbohydrate analyses of the plants of the different batches show the same features as the growth analyses, the maximum carbohydrate contents being found in plants manured with the mixture of two forms of nitrogen on 15th August.

8. The experiments were repeated in the next season by using sodium nitrate in place of potassium nitrate to meet the objection that the increased growth rate and yield of plants treated with potassium nitrate and ammonium sulphate was perhaps caused by the presence of both potash and ammoniacal nitrogen. The beds were also manured with superphosphates before transplantation to make sure that the soil did not lack in phosphates. Exactly the same results were obtained as above.

9. It was then undertaken to determine whether the maximum beneficial effects of early manuring in August were specific for nitrogen fertilizers only or whether the same is true of other manures also. Similar series of experiments were made by manuring plants with superphosphates at different dates and it was found that the greatest increase in yield and the carbohydrate contents were obtained in plants manured on August 10th. As a result of this investigation two facts are discovered (1) the mixture of two forms of nitrogen is a better fertilizer than any one used by itself; (2) the manuring of the plants should be done early in

August or even at the transplantation stage. Late manuring has very little effect on the growth and yield of the rice plants.

The findings of this investigation are now being practically tested in the field and the results obtained in the field will be communicated in a later contribution on the subject.

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SACCHARUM SPONTANEUM L.

A COMPARATIVE STUDY OF THE FORMS GROWN AT THE IMPERIAL SUGARCANE BREEDING STATION, COIMBATORE.*

BY

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(With Plate LXXVI and six text-figures)

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I. INTRODUCTION.

The following investigations were undertaken in order to find out the nature of the different forms of *Saccharum spontaneum*, collected and grown for study at the Sugarcane Station at Coimbatore, with a view to tracing out their relationships and to classifying and grouping them into sub-species, varieties or forms as the case may be. The wild Saccharums have been receiving considerable attention in recent times, as the hope is widely entertained that by employing them as parents, it

* Contribution from the Department of Botany, University of Madras.

might be ultimately possible to breed seedlings highly resistant, if not immune, to the various diseases prevalent in cultivated canes. In the year 1928, an aeroplane expedition under Drs. Brandes and Jeswiet explored the island of New Guinea in search of such types, and resulted in collection of a number of forms, the most important of which is claimed to be a giant form called *Saccharum robustum*.

The need for such a study has been long felt, and though the subject has already been investigated to a considerable extent from several points of view, there are many who still believe that a careful study of the wild forms growing in India and in the Oceanic islands would disclose well-marked groups among them and may furnish a clue to the origin of the three species of cultivated canes. Considering the fact that *Saccharum spontaneum* has a distribution from S. Europe to the South Sea Islands, extends over the entire tropical and sub-tropical belt of the Old World, ascends to an altitude of 6,000 ft. and is adapted to a series of ecological habitats, it is not unlikely that an investigation of the forms would lead one to distinguish groups, possibly species among them. This was felt all the more when several of the forms were collected from different parts of S. Asia and grown side by side at Coimbatore, for the forms so grown showed obvious differences in many respects. Some of the important differences were pointed out by the Government Sugarcane Expert [Venkatraman, 1930], when he also emphasised the necessity for making a close study of the forms. The fact that the Java form when crossed with sugarcane gave seedlings with poor sucrose content and a high degree of resistance to mosaic, the Coimbatore form in a similar cross behaved in quite the reverse manner, yielding seedlings with a higher sucrose content and a low resistance to mosaic, suggested that in their genetic composition the different forms might show interesting and perhaps useful differences. A study* of the forms was therefore regarded as highly desirable as well for its own sake as for its utility in the classification of *Saccharum*, and in the breeding of useful varieties of sugarcane.

An old Linnaean species, *Saccharum spontaneum* as mentioned by Kunth in Enumeratio Plantarum is very restricted, but in its present sense it includes the three Indian species *S. spontaneum*, *S. semidecumbens* and *S. canaliculatum* of Roxburgh. Hackel in his monography of the Andropogoneae describes three sub-species under *S. spontaneum : indicum*, *aegyptiacum* and *luzonicum*. The sub-species *indicum* is divided into two varieties, *genuinum* and *juncifolium*, of which the first has by far the widest distribution in Southern Asia, and includes within its range Roxburgh's three species besides others. Under sub-species *aegyptiacum* are brought together three varieties, from Egypt, Java and Nepal respectively. The sub-species *luzonicum* is restricted to the Philippine Islands.

* As the staff at the Sugarcane Station was engaged solely on the economic aspect of cane breeding, this purely academic question of studying the different wild forms was left to a University, that could bring to bear upon the problem a more detached outlook.

Hole [1911] dealing with the ecological aspects of *S. spontaneum* believes Hackel's var. *aegyptiacum* to be merely one of the several ecological forms of the Indian form. He makes the suggestion that the sub-species *indicum* could be extended to include sub-species *aegyptiacum* and also believes that it is inadvisable that these forms with their intermediates should be separately defined under different names as sub-species or varieties.

In the later attempts at the classification of the genus *Saccharum*, much importance has not been given to the various forms of *Saccharum spontaneum* so far as the available literature shows. Jeswiet in reducing Hackel's twelve species of *Saccharum* to four, distinguishes *S. spontaneum* in the main from the others by the presence of the IV glume, the ciliation of the lodicules and the presence of long underground root-stocks. Bremer [1925] finds different chromosome numbers in the Glagah varieties of *Spontaneum* from Java and Celebes, and adds that this difference is associated with morphological differences between them. He also suggests that if more Glagah individuals with 40 chromosomes become known and described, they may have to be separated as a distinct species.

II. Material and methods.

The present study, it may be mentioned at the outset, is confined to the forms of *Saccharum spontaneum*, which had been obtained from time to time from various parts of Northern and Southern India, Burma and some of the Islands of the East Indies, and as such, is limited in scope. There are about twelve different forms in the collection, including the foreign specimens, and all these have been propagated from year to year by cuttings or "setts". The first plantings had been made by the late Dr. Barber as early as 1911, and the most recent additions, made by Rao Bahadur T. S. Venkatraman, have been there for the past three or four years, and in the course of these years, they have been grown continuously in fields under uniform conditions, receiving the same treatment in cultivation as the Indian canes. They have throughout been under the observation and care of Mr. R. Thomas, who has a personal and intimate knowledge of them; and in the course of these twenty or more years they have not been noticed to exhibit any important change in habit or general appearance as a consequence of the new environmental conditions—a circumstance of some significance to be mentioned hereafter.

The forms investigated were altogether thirteen in number and the names adopted here are those by which they are labelled in the Sugarcane Station. Of the thirteen, the last one was found to be an *Erianthus*, and therefore excluded from this account. No. 9 in the following list is a seedling of No. 8, and was grown separately because it showed a higher sucrose-content. As however, this difference was not accompanied by morphological differences, it has been mentioned

jointly under Dacca. Holes I and Holes IV were reared from seeds obtained from Dehra Dun; they also showed differences in the sucrose-content, but not in their morphological characters. They are jointly referred to as Holes.

The following table gives the available information* about the collected forms :—

TABLE I.

Original habitat, date and mode of first planting of Spontaneum *forms.*

No.	Name	Obtd. in the year	Place of collection	Original habitat	First planted as
1	Lahore . .	1927	Lahore, Punjab . .	Along river-banks.	Sett.
2	Local . .	1911	Coimbatore . .	Elevated places in a tank.	Do.
3	E. C. C. . .	1930	East Chitra Chavadi, near Coimbatore.	Banks of a stream.	Do.
4	Gerah Bon .	1914	Assam . . .	Not known.	Seedling.
5	Rellagadi . .	1915	Godavari Dt., S. India.	Bands near wetlands.	Sett.
6	Holes I . .	1912	Dehra Dun, N. India .	Not known.	Seed.
7	Holes IV . .	1912	Ditto .	Do.	Do.
8	Dacca . .	1914	Dacca, Bengal . .	Ponds.	Sett.
9	M11292 . .	...	(from Dacca form in Coimbatore.)	.....	Seed.
10	Burma . .	1929	Upper Burma, near Mandalay.	Not known.	Sett.
11	Glagah . .	1917	Pasoeroean, Java .	Do.	Do.
12	Sumatra . .	1929	Prapat, Sumatra .	Do. Alt. 4,000 ft.	Do.
13	Gigas . .	1929	Ditto .	Do.	Do.

The work of investigation was mainly along two lines, morphological, both floral and vegetative, and histological including the internal anatomy and the nature of the epidermis. Morphological descriptions and data were taken in the field with as much detail as possible, but it was found unprofitable to take extensive notes of such characters, as for instance, the number of joints in the cane, etc.

* These details were supplied to me by Mr. R. Thomas, the Senior Assistant of the Sugarcane Breeding Station. The maintenance of the various forms and the collection of some of them are largely due to his keenness and enthusiasm.

This in relation to growth and branching has been amply dealt with by Barber [1919] and to some extent also by Hole [1911]. A large number of characters were applied and compared, but showed no or only very insignificant differences and were therefore rejected. But, in general, attention was paid not only to the characters relied upon by systematists but also to those obvious field characters used by the staff of the Sugarcane Station in arranging cane varieties, as far as they were found useful.

A detailed examination of the inflorescences and of the flowers was made under a stereoscopic microscope, and measurements of parts of spikelets made with an ocular micrometer. At first over twenty spikelets of each variety were measured and averages worked out, but as very little variation was observed, the number was later on reduced to fifteen or ten. The number of inflorescences examined in each case was one or two, as most varieties flowered only once, and some produced only one or two arrows.

In the investigation of anatomical structure, permanent preparations from a hand microtome were used. Cross and longitudinal sections of the various parts of internodes at various stages of growth were examined, as well as cross sections of the laminae.

Studies on the epidermis were made from peels of the epidermis, obtained by treating the required portions of the plants with Schultz's maceration mixture, used in similar studies on cane varieties by Artschwager [1930]; but as semi-permanent preparations were required, the material was stained for cellulose with tannic acid and ferric chloride, and for cork with Sudan III and mounted in glycerin. Examination was made in each case of :—

1. Epidermis of a well-developed internode.
2. Epidermis of stolons and rhizomes.
3. Epidermis, both upper and lower, of the leaf-blade.

All epidermal counts were made under the microscope.

III. General description.

(*a*) *External morphology.*—The specimens of *Saccharum spontaneum* grown for study at Coimbatore are tall, perennial, gregariously growing grasses; erect, decumbent, or almost prostrate, tufted or evenly scattered, often provided with long creeping rhizomes; moderately to heavily stooling, attaining a height of 8 to 18 ft., excluding the underground stolons, prostrate culms reaching still greater lengths; varying in thickness from ·2 to ·6 inch. Growth vigorous in some, slow in others. Innovation shoots intravaginal or extravaginal, developed mostly on culms which have arrowed or been destroyed by the moth-borer, less often also on growing shoots. Culms rooting at the lower nodes in prostrate forms or when

flooded with water, usually straight, in prostrate forms and in the subterranean portions of erect forms, curved or bent; very rarely slightly staggering at the nodes; erect forms always straight above the ground level. Fistular in older and full-grown parts, and in the underground stolons; solid in younger portions. Pale green to glaucous green, sometimes deep chrysolite green in colour when young, buff-yellow to maize-yellow and finally bone-white when old but not exposed by the fall of the leaf-sheath; ochraceous tawney to morocco-red or claret-brown if exposed to the sun. On the surface, longitudinally finely parallel-striated on the internodes, owing to the pressure upon them of the veins of the leaf-sheath; polished, lightly to heavily pruinate especially where still protected by the leaf-sheath. Sparsely to pronouncedly silky below the panicle and between its nodes, elsewhere glabrous.

The number of nodes on an aerial culm may be as high as 28-30, but on an average varies within 16-25. Nodes slightly constricted at the insertion of the leaf on the culm and always at right angles to the length of the culm. Internodes terete to subcylindrical, not channelled above the bud, except where the bud has commenced to grow at an early stage; shorter towards the base of the culm, longer above, produced when the growth is vigorous; generally very long immediately below the panicle. Length of the internode from 2 to 5 inch in some forms, 4 to 12 inch in others; internode widest at the growth-ring; below, abruptly narrowed at the root-band, as far downwards as the insertion of the lower leaf-sheath; above the growth-ring, it tapers gradually and slightly towards the top of the internode to dilate again just about half-an-inch below the upper node. The root-band portion thus appears slightly swollen and rounded. Older internodes generally more uniform in thickness. Bloom in the younger internodes deposited only towards the top of the internode, while in older ones, it extends more or less uniformly and profusely all over the internode, being slightly heavier and more persistent at the glaucous band just below the node, and is absent at the root-band.

The growth-ring in younger internodes is even, tender and of a bright grass-green colour, not localised to a narrow band. In older ones it is even or slightly elevated as a narrow ridge; hard and concolorous or a shade more yellowish than the proper internode. The width of the ridge may be as little as ·05 inch in tufted and dwarfed forms, and as much as ·12 inch in prostrate and robust forms. Sometimes unequal in widh all round, being wider on one side than on the other, this being brought about where the culms have bent or straightened themselves secondarily through the activity of the growth-ring.

The root-band varies in width from ·2 inch to ·4 inch and in some cases less than ·2 inch, distinctly lighter in colour than or sometimes concolorous with the

corresponding internode, rarely greenish tinged. Except in fully dry internodes, it is imprinted upon by the veins of the adpressed leaf-sheath.

The characters of the buds were found to be more or less unstable, for though constant in the same clump, they vary in the seedlings. In shape they may be diverse, and possess the characteristic hair-groups often used in the classification of cane varieties.

Though several categories of structures are morphologically equivalent to the leaf-sheath, the term as used here refers only to that borne on a full-grown aerial culm, and possessing a more or less well-sized lamina. Such a leaf-sheath varies in length from 8 to 13 in. and may sometimes be as long as 17 in. Width greatest at the very base, varying from ·8 in. in thin-stemmed plants to 2·4 in. in robust ones, and narrowest at the junction of the sheath and the blade. Sheaths persistent; extending at the base about 1½ times round the base of the internode, sometimes a little more, sometimes a little less, the outer margin overlapping the inner. The overlapping margin is alternately the right and the left one in successive nodes. The inner margin at the node reaches close to or within half-an-inch of the bud. The sheath is coriaceous and thickest medially, particularly just above the bud, becoming gradually thinner towards the margins where it is delicate and membranous. A circlet of cilia may be present at the base as in some forms, or indistinct or absent as in others. In the robust forms the margin of the sheath is ciliated towards the top. The sheath in some is glabrous, in others covered with a thin vestiture of spiny hair, which may be persistent or early deciduous. In the Dacca form hair is present in the lowermost sheaths, the later-produced sheaths being glabrous. The vestiture of hair is absent in the youngest sheaths; in older ones which are still protected by the lower sheaths, the hairs are adpressed upwards. The hairs stand out when the sheath is exposed.

The sheath may be green, partly flushed purple or quite purple, in some cases purple interiorly at the base, rarely glaucous. In some forms all the sheaths are green, while in others, the early-produced sheaths are purple, later ones green.

Leaf-sheath purple	E. C. C.
Early sheaths alone purple . . .	Sumatra. Glagah. Dacca. Burma. Rellagadi.
Leaf-sheaths always green . . .	Local. Holes. Gerah Bon. Lahore.

The throat is glabrous or slightly pubescent behind the ligule in some and bearded and lanate in others. A line of moderately long cilia may be present be-

hind the ligule as in Dacca. On either side of the ligule or just behind it is a tuft of long cilia, which is noticeable above the shoulder of the leaf-sheath on either side. These tufts may be more or less conspicuous, in some more than in others, but are always present, and where marginal cilia occur on the sheath, these tufts are continuous with them.

The ligule is deltoid to roundedly triangular in some and truncate or crescent-shaped in others. In the former the length along the median line varies from ·08-in. in Lahore, to ·3-in. in some of the more robust prostrate forms; this vertical length is more or less equal to the width of the ligule at its base, with only slight departures. In the second type of ligule, the length along the midline varies from ·08 to ·13 in., but the width of the curved base is three to six times this length. The basal line of the deltoid ligule is almost straight and slightly depressed in the middle and at either end in some; in the Lahore form the ligule is reduced in size and the depressions of the basal boundary are approximated. In the crescent-shaped or truncate ligule the basal border is widely curved with a slight depression in the middle. The ligule is glabrous and polished on the adaxial surface, finely ciliate or stipate dorsally, the cilia being minute and adpressed. The margin is finely and shortly ciliate in the early stages, but later fimbriate and often brokenly indented and scarious. Chartaceous to membranaceous, hyaline to opaque, pale white to pale-grass green, but early discoloured to brown.

Collar is the name given to that part of the abaxial surface of the leaf where the lamina joins the sheath. This region is invariably marked, distinctly in some, and indistinctly in others by two triangular, rarely almost semi-circular areas, the apices of the triangles almost meeting over the abaxial surface of the midrib. They are called the "transverse mark", or "ligular bands". They may be yellow or bright green or sometimes discoloured to brown; glabrous, later sometimes slightly glaucous.

While all the sheaths on an aerial culm generally bear a lamina, this is by no means always the case, for no lamina is borne by those sheaths at or near the ground level, or by the lower scales of the innovation shoots. The description given below, therefore, refers only to the prominent leaves of an aerial culm which is yet too young to flower.

The number of such leaves on an aerial culm is about five, ranging between four and seven, and only in the case of Gerah Bon it is well over seven. The number of leaves is more or less constant throughout the life of the culm. The average length varies from 2 ft. in some prostrate forms to 4 or 5 ft. in some of the tufted ones, and may sometimes be as great as 6½ ft., ·3 to 1 in. in width at the widest portion. In the Lahore form the lamina is reduced to the midrib, and is thus only ·1 in. in width, the width of the midrib,

The leaf-blades are long, linear, narrow to very narrow, tapering towards the apex, which ends in a long fine point; gradually narrowed from the middle towards the base. In some forms the lamina is reduced to the midrib at the joint between the lamina and the sheath; in others a considerable portion of the lamina is present on either side of the midrib at the joint. Generally gently curved along the entire length, except in Lahore, where it is erect and stiff. Sea-green to bright grass-green in colour, subcoriaceous, rigidulous, subscurfy to scurfy towards the apex on the upper surface and smooth towards the base and on the lower surface; margins scabrid, except in Lahore. Midrib prominent, white or lighter in green than the lamina, concavely channelled on the adaxial surface, and deeply convex below; widest at the base, gradually tapering towards the apex, percurrent but absent at the very apex of the lamina, ·1 to ·25 in. wide.

The inflorescence is a panicle, varying in length from 8 in. in some specimens to 2½ ft. in others, and in width from 6 in. to 12 in. at base; generally lanceolate to conical when fully spread out, but in some cases also oblong-conical or oblong; borne well above the leafy clump by an elongated central axis. In colour pale or greyish white, cream or golden brown, greyish brown or rarely purplish grey, spotted when the glumes are red-blotched or when the purple-coloured stigmas are protruded, and almost white to silvery white in the fruiting stage. In texture soft and downy, especially where the callus-hairs are long and profuse. Main axis always silky-pubescent below the panicle, more so in some than in others; may have a diameter of from ·08 in. in some to ·2 in. in others, the stouter axes not necessarily being borne by the more robust forms. Terete below the panicle, but ridged between its nodes, the ridges being caused by the secondary axes which are folded upwards and pressed against it during the so-called "boenting stage", that is when the inflorescence is still enclosed within the sheaths of the flag-leaves. In this region the axis is sparsely silky-haired.

The secondary axes are in whorls on the main axis, and when the inflorescence is fully spread out, they spread outward almost at right angles to the main axis. They are more or less pulvinate at base, and are not all inserted on the main axis at the same level; indeed, in some cases they may be irregularly distributed in the region of the node. The longest racheae are near the base, but the lowermost racheae are very short in a lanceolate panicle. In some forms the long basal secondary axes bear branches of the third order. Racemes short-jointed, length of the joint rarely exceeding ·25 in. Joints filiform, fragile, especially in the fruiting panicle, straight or rarely bent to accommodate the spikelets, in which case the raceme is pronouncedly wavy to sinuous. Each joint is enlarged at the upper end, slightly cupular at the top, and rounded at the lower end, and bearing scattered hairs on the surface.

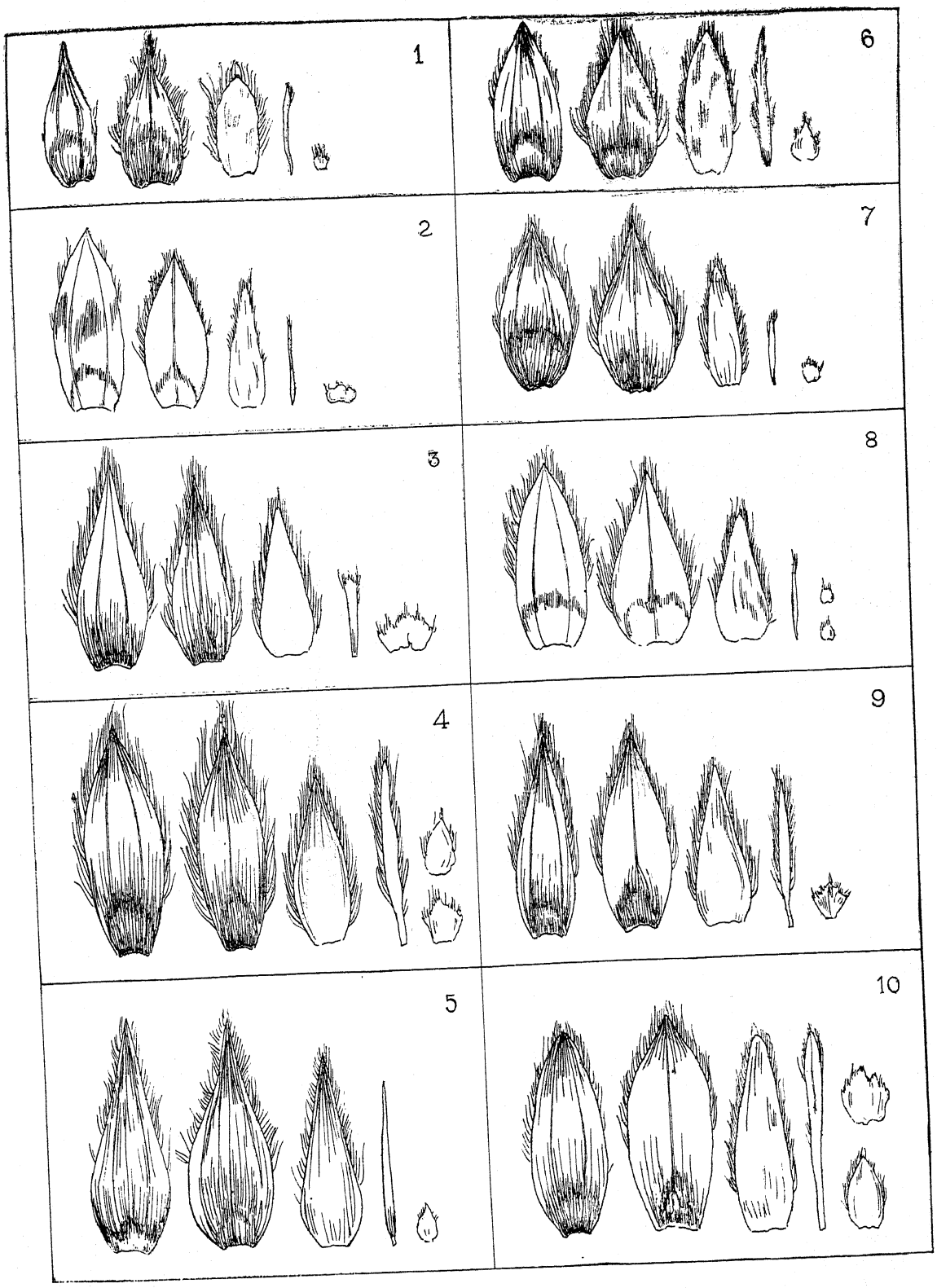
The spikelets are invariably binate, one of a pair pedicelled, and the other sessile. The pedicel varies in size as does the joint and the pedicelled spikelet may be at different levels in relation to the sessile one. There is considerable variation in the same panicle with regard to the density of aggregation of spikelets, different lengths being attained by joints in different parts of the same panicle and at different stages of development.

The pedicelled spikelet at the growing stage is often slightly bigger than the sessile one, and is always the first to develop and fall off from the pedicel. Later the joints themselves are disconnected from one another, and the sessile spikelet falls away with the corresponding joint and pedicel.

In shape each spikelet is lanceolate, sometimes oblong-lanceolate, subulate, roughly triquetrous; in colour light or cream or yellowish brown, sometimes, as in Burma, purplish grey. In most of the Indian forms the spikelets have a scarlet blotch or band across the middle, the base and apex being yellow or white. In length, the tiniest spikelets are ·08 to ·1 in. and the largest ·16 to ·19 in., with all intermediate sizes between these extremes, the size of the spikelet being independent of the size of the plant. Callus hairs are silky white, rarely violescent varying in length from ·35 to ·77 in., or 2 to 6 times as long as the spikelet, or even more.

The I and the II glumes, are almost equal in length, lanceolate to ovate-lanceolate, sometimes oblong-lanceolate, (II glume rarely elliptic or ovate) acute to acuminate, subulate, sometimes mucronate, margins subentire or ciliated to various extents from the apex downwards; chartaceous to hyaline above, and subcoriaceous towards the base. At the base generally is a coriaceous yellowish patch; in some forms, Local, Lahore, etc. the first glume is a subgibbous in the lower half, and the second glume sometimes deeply keeled. The first glume is 2-veined and folded along the veins, the folded margins overlapping those of the II glume, the two veins forming the two angles of the triquetrous spikelet. The II glume has a single median vein and is keeled along it, the keel forming the third angle. The ciliated margins are often again inflexed inwards about ·004 in. from the margin. The length of the I and the II glumes corresponds to the length of the spikelet, being nearly equal to it and in width they vary from ·035 to ·08 in., the II glume being slightly wider than the I.

The III glume is shorter than the I or the II, lanceolate, rarely oblong-lanceolate, acute, marginally ciliated about $\frac{2}{3}$ of the way down from the apex, often down to the base, very rarely subentire; hyaline, nerveless, ranging in length from ·06 to ·13 in., and in width from ·012 to ·052 in. The IV glume is always linear, slender, narrowed towards the insertion, ciliated to various extents, hyaline, nerveless, rarely faintly one-nerved, varying in length from ·028 to ·19 in. and in width from ·001 to ·016 in.



I, II, III & IV glumes and paleæ of *Saccharum spontaneum* forms drawn to the same scale.

1. Gorah Ron. 2. Burma. 3. Bellagadi. 4. Dacca. 5. E. C. C. 6. Sumatra. 7. Lahore.

The palea is small to minute, variable, more or less irregularly suborbicular to quadrate, or ovate, hyaline, generally ciliated slightly on the upper margin. Lodicules are cuneate, thick and opaque, pale white or red-tinged in some forms, concave on the upper margin and ciliated sparsely on the apices.

Stamens are three in number, and when fully developed, about ·08 in. in length; usually yellow in colour, in Lahore orange-red. The ovary is minute, and the style has two purplish-coloured brushy stigmas about ·06 in. in length.

(*b*) *Anatomy.*—The anatomy of sugarcane has been dealt with in detail by Artschwager [1925] and others. An account of the anatomy of *Saccharum spontaneum* would be a recapitulation of these accounts, as spontaneum does not seem to differ to any large extent from the other *Saccharums* in this respect; nor is there any mentionable difference in the broad features of the internal structure of the stem between the various forms of spontaneum, notwithstanding that in habit and external morphology they differ markedly from other species of the genus and among themselves.

The topography and the essential structure of the stem-epidermis is in agreement with the type of structure which obtains in grasses in general, for which considerable literature is enumerated by Prat [1932]. In the investigations of the features of the epidermis, the characters used by Artschwager [1930] in classifying varieties of cane were at first applied, but it was impossible to say that any reliance could be placed on them when applied to the spontaneum forms. Artschwager's criteria were mainly the following:—

1. Width of cells.
2. Presence of pointed cork-cells.
3. Number of short cell groups for a given field.
4. Number of solitary cork-cells.
5. Number of stomata.

With regard to the first character the number of cell-rows per mm. was found to vary from 50 to 100; and though the different forms to some extent showed different widths, yet there was so much overlapping in the range of width of each that it was not possible to draw a line between any two. Burma was seen to possess the narrowest cells.

Pointed cork-cells are present in Burma and Dacca and rarely also in Glagah. The number of short-cell groups may be anywhere between 105 and 365, and like the width, it does not permit of any line of demarcation. Besides, in some forms it is extremely variable, being almost entirely dependent on the (more or less varying) length of the long cells. The presence and the number of solitary cork-cells is also variable and the distribution of stomata often erratic.

Several other features of the epidermis were compared as far as was possible, but the differences were found to be either inconstant or intergrading, there being

no demarcation of any form or forms from the rest. One feature *viz.*, the proportion of silica-cells to the number of short-cell groups, at first seemed to suggest a distinction between the Indian and the East Indian forms. The former seemed to possess mostly cork-silica pairs, and the latter mostly single, paired or grouped cork-cells. On greater scrutiny however, this difference was found to be less constant than was first supposed, though one form, Burma, was unique by the fact that practically no silica cells were to be met with in its epidermis.

The leaf-epidermis on the whole presents a great amount of structural detail, and as in the case of the stem-epidermis, no attempt has been made here either to give a thorough description of the epidermis, or to enumerate the proportions of its various constituents. One of the outstanding characters, however, presents a point of importance.

Some of the Spontaneums differ from the rest in the possession of stomatal grooves on the lower surface of the leaf-blade. These grooves, where they occur, run longitudinally along the length of the lamina and are present all over its lower surface. They are situated between the bands of sclrenchymatous cells which abut on the epidermis above and below the bigger vascular bundles of the leaf. They bifurcate or unite with one another in their course along the length of the leaf, but not frequently. Each groove is formed by about seven rows of cells and the invagination may be deep or comparatively shallow. Both the rows of cells forming the borders of the groove possess toothed or claw-shaped cells alternating with the ordinary short-cells. In the groove may be one, two or rarely three rows of stomata, which always alternate in adjacent rows. Many of the silica cells in the groove bear long hairs of two or three superposed cells. The grooves are best seen in a cross-section of the leaf, and the teeth in a peel of the epidermis; both may, with a little care, be distinguished in the living leaf under a stereoscopic microscope. Of the forms examined only two were found to be characterised by the absence of grooves—Burma and Sumatra.

It must, however, be mentioned here that the epidermis may be studied on a still more extensive scale, taking into consideration the topography and the general structure of the epidermis in relation to the various parts of the plant at various stages of development, but it is to be doubted whether so laborious a study will disclose any marked differnces among the members of a group so compact as the Spontaneums.

IV. Classification.

An examination of the vegetative characters of the Spontaneum forms reveals the presence among them, of two well-marked groups, which correspond to Hackel's sub-species, *indicum* and *aegyptiacum*; but they are distinguishable from one another

solely by their vegetative and not by their floral characters. Hackel's distinctions based on floral characters do not seem to hold in most of the cases, for they show, surprisingly enough, considerable uniformity throughout the series. The vegetative characters, including those used by Hackel, on the other hand, permit of the separation of the Indian Spontaneums from the rest. It is also possible to further subdivide the groups, and here one finds sharp distinctions and is faced, if at all, with exceptions rather than "intermediates" or "transitional forms".

In all about 60 characters, floral and vegetative, were examined. The following table gives the selected characters and the groups of forms in summary.

TABLE II.

Classification of the Spontaneum forms.

No.	Character	Indian forms	Dacca	East Indian forms including Burma
1	Habit . .	Tufted or prostrate (except Rellagadi).	..	Erect.
2	Ligule . .	Deltoid	..	Crescentic.
3	Lamina . .	Narrowed almost to the midrib at the base.	..	Not so narrowed.
4	Sheath . .	Glabrous	..	Hispidulous.
5	Stomatal grooves .	Present	..	Absent (except glagah).
6	Throat . .	Slightly bearded. Rarely glabrous.	..	Always glabrous.
7	Culms . .	Slender	..	Robus
8	Width of leaf .	Narrow	..	Wide.
9	Leaf-module .	Over 100	..	Less than 60.

A glance at Table II will indicate that the first six characters distinguish the Indian Spontaneums from the East Indian. Dacca also falls in with the Indian forms, but agrees with the East Indian forms in the last three characters. The value of these characters and the position of Dacca will be considered later.

Burma agrees with the East Indian Spontaneums in all the characters mentioned in the table; but there are several important features which distinguish Burma from the East Indian forms, Glagah and Sumatra. Indeed, Burma can be singled out as a distinct type by itself, possessing peculiar features of its own. Its height far exceeds that of all other forms and the culms are also more robust than those of any other. It has a yellow-green colour when young and is later amber-brown to russet, whereas the other forms are somewhat sea-green at first and maize-yellow when fully grown. Bloom is absent or very slight. The internodes almost always outgrow the sheaths, which is never the case in other Spontaneums. The individual spikelets and the inflorescence as a whole are purplish grey, whereas in the others they are yellowish brown or dusky yellow. It has an extremely minute IV glume, smaller even than the IV glume of the Indian forms. Furthermore, its epidermis is constituted with few or no silica cells, and the cell-rows have the smallest width among the forms examined, though how far these epider-

mal characters are reliable is doubtful. All these features admit of separating Burma from Glagah and Sumatra, but it has several important characters in common with them. It may be noted that Burma has no characters in common with the Indian Spontaneums and is in no way intermediate between the two main groups, as its geographical position would lead one to imagine.

V. Discussion.

It is best to primarily examine and estimate the value of the various charaters, in order to find out how far they are reliable in tracing out taxonomic relationships.

(*a*) *Inflorescence.*—The floral characters have always been regarded as the most important of all, giving the most reliable data and establishing the most fundamental relationships. While exhibiting individually very small differences in floral parts, the inflorescences of the various forms have, without exception, all the characteristics of *Saccharum spontaneum*, not deviating from the descriptions given in floras. On the contrary, the range of forms under observation was considerably shorter than the scope of the entire species, which, according to Hooker, includes forms in which the IV glume is always absent,* whereas in all the forms examined, the IV glume was always present, varying among the forms, in shape and size. It was seen, however, that no groupings could be made among the spontaneums based upon the characters of the inflorescence, though extensive notes and measurements were taken on the floral parts. Hackel bases at least four of his distinctions between his sub-species, upon the characters of the inflorescence and of the spikelet, as follows :—

Table III.

Floral characters recommended by Hackel for distinguishing between sub-species. indicum *and* ægyptiacum.

Floral part	Subsp. *indicum*	Subsp. *ægyptiacum*
Racemes	Slender	Robust.
....	Lax-flowered	Dense-flowered.
Spikelets	3-4 mm. long . . .	4-6 mm. long.
Ratio H/S (Hair/spikelet) . .	4-6	2 or sligtly more.

But none of these characters were found to hold true among the forms examined and to have any correspondence with the vegetative characters. The local form had more or less dense-flowered racemes, while in Burma the inflorescence was

* Indeed, Hooker holds that the IV glume is usually absent, but it has been invariably present in all the spontaneums studied, though developed to various sizes. Hole, too, while admitting that it is sometimes absent, suggests the possibility that it may always be present, but overlooked in the dissection of fruiting spikelets. According to Jeswiet also, the only *Saccharum* species in which the IV glume is absent is S. *officinarum*. The probablity, therefore, is that the IV glume is always present in *Saccharum spontaneum*.

comparatively lax and the racemes not quite robust, Sumatra had about the shortest spikelets in the whole series, whereas Dacca and local had spikelets as long as any of the *ægyptiacum* group. The length of the callus hair also showed similar, though not parallel, differences.

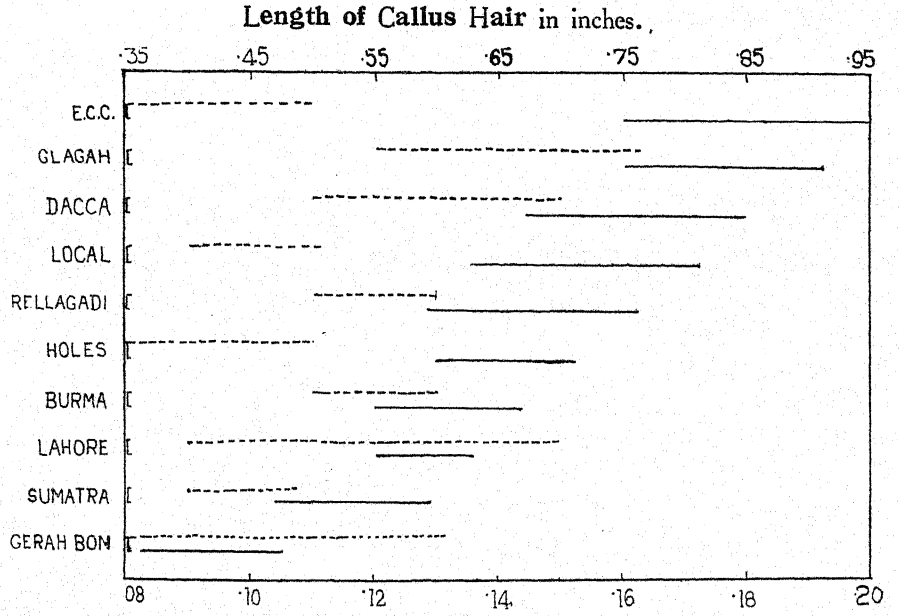


Fig. 1.—Range in size of spikelets of various spontaneum forms. (Dotted lines denote the length of callus hair.)

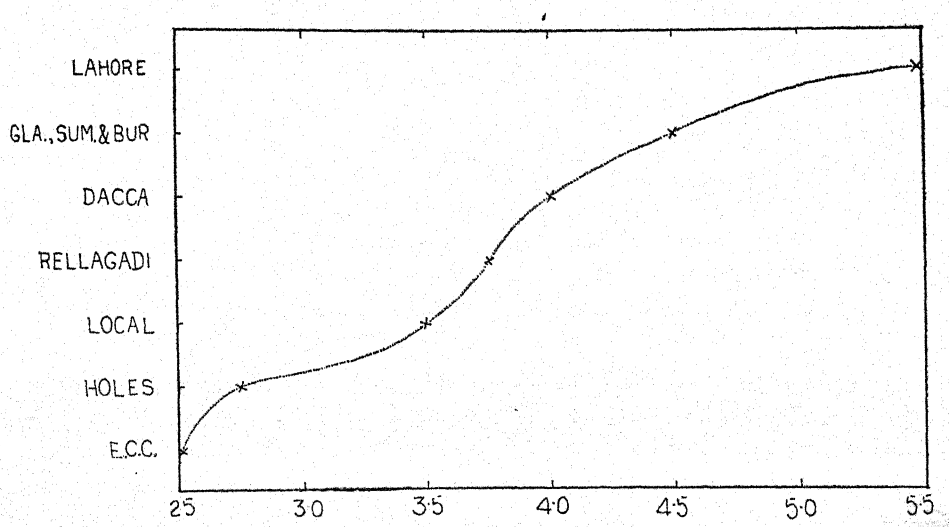


Fig. 2.—Range of H/S ratio in *Saccharum spontaneum* forms.

This condition has been represented graphically in Figs. 1 and 2. The graphs show not only the extent of variation in the whole series, but also in each form. As a matter of fact, the range of variation of each form is probably wider than is represented here, for these graphs have been drawn from measurements taken from a limited number of arrows obtained from a single generation of plants. Hence it cannot be concluded that each form is restricted within its own limits as shown by the graphs though it is not unlikely. But even as it is, the forms present a gradation of characters and sizes from one extreme to the other. The same more or less is the case with characters pertaining to the shape, colour and texture of the spikelets and their respective parts, and also of the inflorescence as a whole.

It has not been possible to find out in what manner cultivation in a clay loam has affected the characters of the inflorescence owing to the fact that the original forms were not available for comparison; but the fact that no distinction can be made according to the floral characters finds confirmation in Hackel's own recognition of the occurrence of intermediate forms. The same has also been the experience of Hole, who states that the difference between the Indian and African forms examined by him is very slight and fails in the case of some African specimens. However, he attributes this to the effects of the environment, particularly that of available moisture. If this were the case, the result of bringing the forms into identical conditions would be to minimise variation. The graphs on the other hand (in spite of the fact that they do not represent the entire variation) indicate that the extremes are as far apart from one another as those mentioned by Hole or Hackel. It seems evident that each form has a definite range of variation of its own (identical with, or greater than that shown in the graph), of which only a part, if at all, is due to environmental factors and that within the limits of the species, there are numerous races and forms, with minute differences in the floral characters, which are so graded that no demarcation of any forms is possible; even those forms, which according to the floral characters are farthest removed from one another have no supplementing differences, either in vegetative or other floral characters or as ecological or geographical races.

(*b*) *Vegetative shoot.*—In the vegetative characters, however, a marked difference is noticeable. There are at least four important characters according to which the species may be subdivided, and these four are not only sufficiently good technical characters but are concurrent mostly with one another, only two exceptions having been noted. These characters may now be examined individually, leaving the paragraphs on the habit to the end.

1. The ligule is generally regarded as a more or less conservative structure, an organ of ancient origin, without any tendency to variation and not subject to modifications under the influence of environmental factors, as most of the structures of

the vegetative body usually are. Two types of ligules are discerned and the difference between them is clearly pointed out in the general description of the forms. They are shortly described as deltoid and crescentic respectively, and the exact shapes and relative sizes may be made out in Fig. 3.

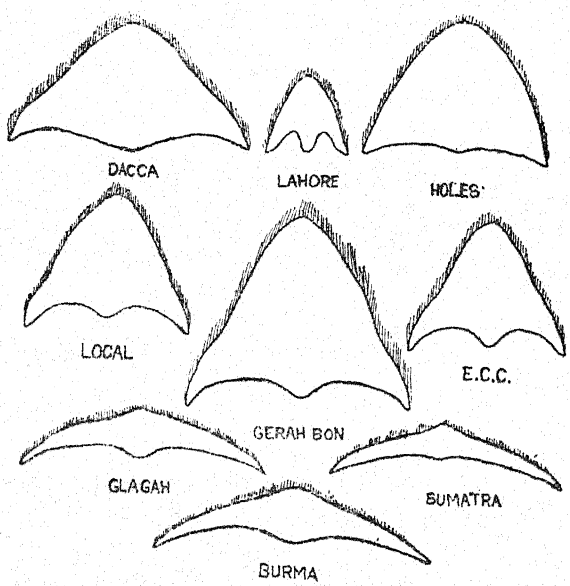


Fig. 3.—Ligule of the different forms of *Saccharum spontaneum* drawn to scale.

The well-marked difference between the two types of ligule is obvious at sight, and there are no intermediate shapes in any of the forms observed. This ligular distinction, simple though it is, is of considerable import, not only because of the conservative and "non-plastic" nature of the ligule, but also because the crescentic ligule of the East Indian forms is an intermediate between the deltoid ligule of the Indian spontaneums and the large, narrow, band-shaped ligule of the other species of *Saccharum*, *viz.*, *S. munja*, *S. arundinaceum* and *S. officinarum*, while in *S. narenga* again, the ligule is of the deltoid type. The significance of this ligular distinction, therefore is more far-reaching than is apparent and may throw light on the inter-relationships of the different species of *Saccharum*.

2. The characters of the lamina have been used by Barber in his studies no less than other characters. Indeed, the differences in the leaf constitute the most outstanding difference in the general appearance of any two plants. It seems, however, certain that some of them are not as reliable as the characters of the stem or of the ligule. Of the large number of leaf-characters recommended by Barbar, only two have been found useful in these studies—the width and the leaf-

module. Much has been written on the apparently minor character of the drooping of the leaf-tip, but grown under identical conditions, all the spontaneums (except Lahore) had widely curving leaves, and though the leaves of some forms were stiffer to the touch than others, it was impossible to make any sharp distinction between the forms based on this distinction. The following points may be noted here :—

1. Lahore has erect and stiff leaves (reduced to the midrib).
2. Though wider leaves as a rule are more pliable than others, the leaves of Dacca differ from the other wider-leaved forms in that they are more coriaceous, resembling in texture such Indian forms as Holes or Gerah Bon.

In the width of leaves considerable difference was found, the Indian Spontaneums possessing a narrow lamina with the exception of Dacca. From the descriptions of Hole and Hackel it is evident that the East Indian Spontaneums are broad-leaved, and that in India both broad-leaved and narrow-leaved varieties occur ; but both of them treat the character with suspicion as dependent largely on habitat. Comparison of the experimental forms with the available original forms has shown that the width of leaves is influenced by the habitat and by the stage of growth only to a limited extent, and that carefully applied, it is a reliable character. The increase or decrease in width due to the moisture content of the soil, if any, is quite within narrow limits. The 'dry-sand' from Lahore was grown for years in a moist soil, but its leaves are still wiry and consist only of the midrib. No amount of moisture could induce E. C. C., a stream-bank form, to put forth leaves even half as wide as Dacca. Further, it may be pointed out that the forms generally found growing along the sandy banks of monsoon streams are, for a part of the year well-irrigated by the streams ; but for the rest of the year, when the streams dry up, the clumps grow in sand, and under these seasonal changes, they are not noticed to exhibit any large periodic alteration in the width of their leaves.

It was possible to distinguish the following three undoubted classes among the Indian forms, based on the width of leaves :—

Lamina broad	Dacca.
Lamina narrow	Local E. C. C. Rellagadi. Gerah Bon. Holes.
Lamina reduced to midrib	Lahore.

and one class among the East Indian forms, the leaves of all being broad.

The length of the leaf is another character frequently used, but neither the length nor the width may be as reliable as the leaf-module ; for in the spontaneums the leaves produced in the vigorous growing season are slightly longer and wider than the leaves produced in later life, when a large number of small shoots grow on the parent culms or on the stolons and arrow. In such cases, only the leaf-module (the ratio of the width of the leaf to its length) remains more or less constant throughout. In the forms investigated, the leaf module of the East Indian forms is nearly twice that of the Indian forms. While in the former the length of the leaf is less than 60 times the width, in the Indian Spontaneums (excepting Dacca) the corresponding number is over 100. One more point may be noted here ; E. C. C. and Rellagadi which are short-leaved, show differences from the other Indian forms if the length alone is considered ; but they quite fall in with the Indian Spontaneums when the leaf module is taken into account.

The narrowing of the lamina at its insertion on the sheath is an important diagnostic character. This character has been used by Hackel in distinguishing var. *genuinum* from var. *juncifolium* and the lamina of var. *aegyptiacum* has been mentioned as hardly narrowed at base. The character seems to be one of more general application, however ; for all the East Indian forms could be distinguished from the Indian forms by this one character. The Lahore form (*juncifolium*) again, need not be separated from the true Indian forms (*genuinum*) on the basis of this character. It possesses a lamina reduced all along its length to its midrib and it is interesting to reason out whether the lamina was originally of the Indian or the East Indian type. The " narrow-inserted " laminae are always associated, and probably correlated, with the deltoid ligules, and the fact that Lahore possesses a deltoid ligule certainly indicates that its laminae were originally narrowed at the insertion.

One unmistakable feature in which the leaves of *Saccharum munja*, *S. arundinaceum*, *S. officinarum* and species of *Erianthus* differ from those of the Indian Spontaneums is the nature of the transition region between the leaf-sheath and the lamina. In the Indian Spontaneums this region is very short and sharply marked off, and the leaf at its insertion on the sheath is reduced almost to the midrib, which alone, lower down, widens out into the sheath. In the other extreme type, *viz.*, the Munjas, Arundinaceums, etc., the narrowing at the insertion is not so great, and a considerable extent of the " blade-region " remains on either side of the midrib at the insertion. Beyond the joint or the insertion, the midrib is flattened out into the middle region of the sheath, the " blade-portion " merging into the sides. As a result of this no angle is formed by the lamina with the sheath ; the former gently curves out from the latter and the ligular bands (the " transverse mark ") are absent. In the East Indian forms, the lamina does make an angle with the sheath, but it is not so narrowed at base as in the Indian Sponta-

neums, and the midrib is less than half the width of the lamina at the level of the insertion.

It will be seen that this character is closely related to the shape and to the extent of the basal line of insertion of the ligule. The ligule is horse-shoe shaped in those forms in which there is no fold or angle at the junction of the lamina and the sheath as in *S. munja*, *S. arundinaceum*, etc. The wider the insertion, the more band-shaped the ligule.

3. It is difficult to say how much importance should be attached to 'stomatal grooves'. It is literally a microscopic character, but the distinction is clear and seems reliable. It cannot be conceived of as a feature developed or discarded as the result of a change in environment. Xerophilous forms grown for years in moist soil still continue to show the stomata in grooves. There are no other features of the leaf associated with this character. This, for example, is the only vegetative character by which Glagah can be distinguished from Sumatra. The fact that it occurs as a difference between two otherwise similar forms may suggest that the character is of no significance ; but on the other hand, it may also indicate that it is an extremely stable character which has survived the others. The latter explanation seems to be more probable, as Glagah differs considerably from Sumatra in its floral characters. Among the East Indian forms stomatal grooves are present only in Glagah, and the probabilities are that it is a constant feature of the Indian Spontaneums.

4. The habit may be tufted, prostrate or erect, and as was, pointed out in the general description, the forms of Spontaneum grown at Coimbatore can be classified according to this character. The distinctions can be easily made out in the resulting clumps by even a casual observation.

The first is the tufted form. The plants of this form are bushy and thickly clustered together. There is no tendency to spread. The first erect culms are immediately followed by shoots, which arise from the under-ground or above-ground nodes of the initial culms. The early nodes are close and crowded, the internodes not having elongated. Most of the buds sprout early into activity, and push upwards parallel to, and well in contact with, the parent culm. A repetition of this in the case of the daughter culms results in a more or less dense clump. The tufted nature is due to the fact that the bud starts vertical growth from almost the very commencement and there is no tendency to creep along or under the ground, beyond what is strictly necessary. This is exemplified in Lahore (Fig. 4) and may also be discerned in Gerah Bon. In Lahore as more and more short culms grow out from the base, the top becomes crowded with leaves, and the closely packed clump naturally assumes a hemispherical outline with shoots grow-

ing radially outwards, and as far as space permits, upwards, all round from a limited base.

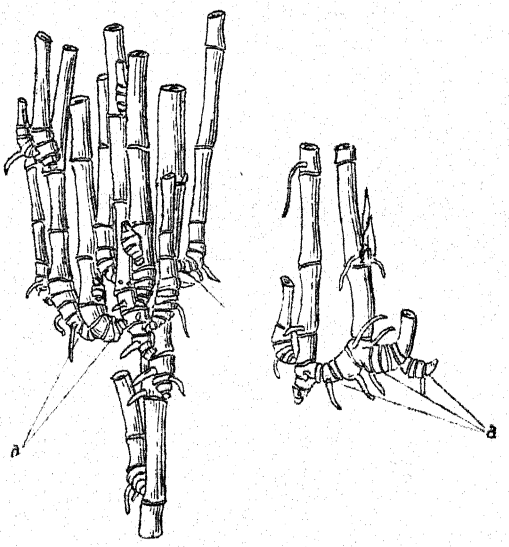


Fig. 4.—Part of a dissected stool of Lahore form.
a. Shoots bending round the base of the mother culm.

The growth here is monopodial ; but the difference in size between the main culms and their basal axillary shoots is soon lost, on account of the precocious activity of the buds, for they grow closely after the parent culms.

Secondly, there is the prostrate or the decumbent form. The first shoots from seed or sett are observed to start upright ; but the later shoots, however, gradually lie across, the growing apical portion alone being curved upwards, and reaching about 3 to 6 feet above the ground. As the upper part of the stem grows upwards, the portion immediately below, as it matures, inclines and becomes prostrate, so that the entire plant never grows beyond a certain height except in arrowing, though it may extend to a great distance outwards in all directions. Axillary buds on the prostrate branches may commence growth, but they soon come to lie flat like the parent culm. The result is a dense maze of long and tortuous culms lying

one across the other, budding and rooting at the nodes, spreading out in all directions, the apical portions alone being directed upwards and outwards.

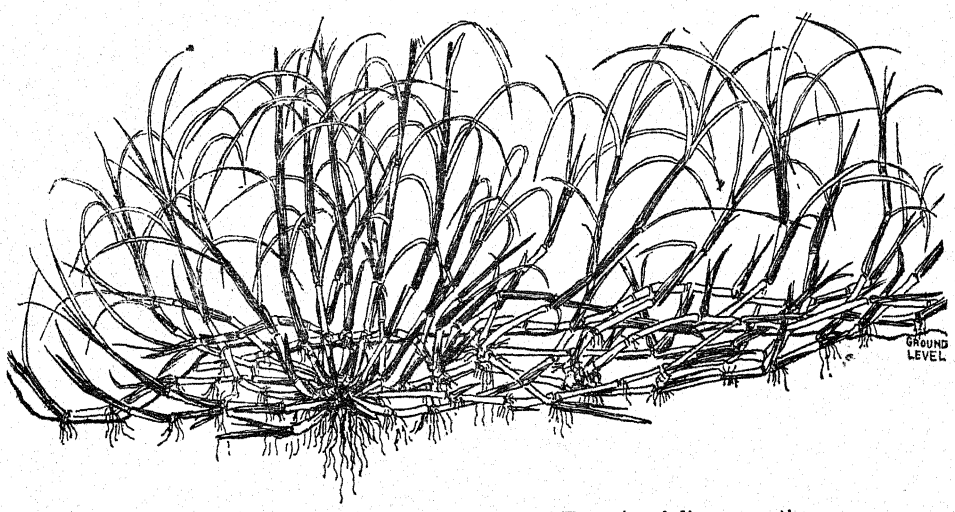


Fig. 5.—Part of a dissected stool of the Local Form (semi-diagrammatic).

The growth here again, is monopodial. The axillary buds sprout into activity considerably later than the parent culm, though they all flower at the same time during the flowering season. Underground shoots are not quite lacking, but they soon appear above the ground and thenceforth commence the decumbent mode of growth. This form of growth is exemplified by Local and E. C. C. In Dacca and Holes, this tendency is not fully expressed, and though in the growth during the second year the prostrate character is more definite, yet they are more or less intermediate between the tufted and the truly prostrate forms.

Finally, the erect form is characterised by the fact that in no portion of the plant is a distinct, sub-aerial, prostrate portion visible. The mode of growth is as follows : The first culms from seed or sett commence growth vertically and remain so throughout. The first few buds on the subterranean nodes grow upwards in the proximity of (but not in contact with) the parent culm. The later buds soon assume a more or less horizontal growth below the level of the ground and grow on till they are far beyond the central culms. The buds borne on this elongated rhizome either grow out and commence a sub-aerial vertical growth or creep underground like the mother-culm bearing them. Several daughter-culms may arise where the nodes are close together, but they never shoot upwards as a clump. Sometimes the migrating tip of the underground mother-culm itself turns sharply upwards and commences thenceforth to grow vertically into the air ; and then the axillary bud at the point where the rhizome has turned upwards continues

the further horizontal, subterranean, outward growth, thus forming a sympode. The result is a large number of upright shoots, separated from one another by considerable intervals of space, and characterised by the total absence of sub aerial, prostrate culms, and having a perceptible tendency towards the sympodial mode of branching.

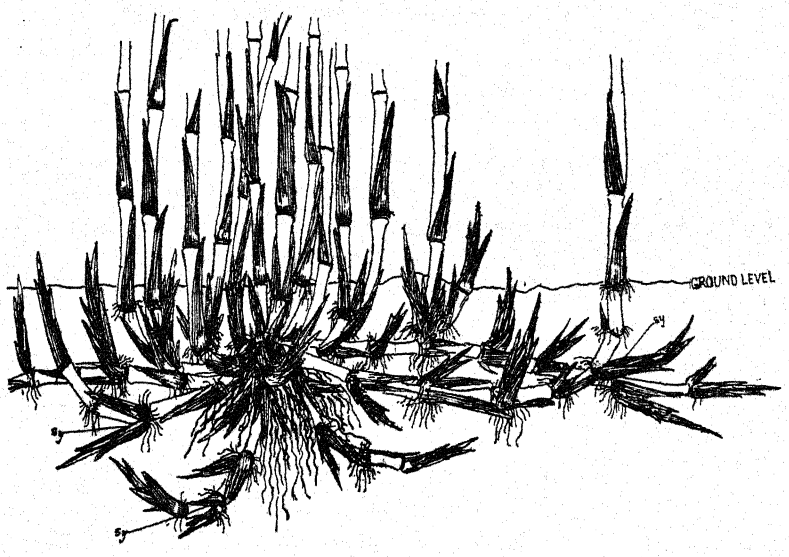


Fig. 6.—Part of a dissected stool of the Burma form (semi-diagrammatic).

Sy. sympodes.

There exists in the previous literature some confusion upon this point, as to the habit and the nature of branching in the Spontaneums and the other species of *Saccharum*. Hole [1911] in describing the modes of growth of Spontaneums has asserted that in (Indian ?) Spontaneums "there is no well-defined rhizome". According to the generally accepted notions about the rhizomes of grasses, a true rhizome would be an *underground* stem in which the mode of branching is strictly *sympodial*. The idea implied in the principle of the sympodium is that the main axis should terminate growth and the further growth be carried on by the axillary bud immediately below. In the rhizomes of grasses, however, it is not a termination of growth, but for the time being, it is only a change in the direction of growth. Such sympodes are met with in the East Indian Spontaneums, especially in Burma, and as such these long underground root-stocks may be called rhizomes. But Hole contrasts *Saccharum spontaneum* with *S. munja* (or *S. narenga*) and describes a rhizome in the latter which is lacking in the former. In these forms (*S. munja*, *S. narenga*) the axillary shoot springs up so close to and so in contact

with the main axis, that it may rather be described as monopodial, the extremely short horizontal portion being necessitated by the fact that in order to grow upwards the axillary bud must get clear of the enlarged base of the mother-culm. Once it is beyond the circumference of this swollen base, it shoots upwards almost in contact with the parent culms. It is a true monopodial branching modified for the mutual spatial adjustments of the culms. This kind of growth can often be seen in a dissected stool of a true tufted Spontaneum, such as Lahore (Fig. 4). On the other hand, there is greater reason to regard the creeping root-stocks of the East Indian Spontaneums as rhizomes, because they are, by nature, underground and have a tendency towards the formation of sympodia. Even in the Indian Spontaneums some (Rellagadi) possess long, underground root-stocks and it would be a mistake to regard Spontaneums as having no well-defined rhizomes and at the same time treat the tufted forms like Munja and Narenga as rhizomic or sympodial.

Barber's observations [1919] in this connection have been more accurate. He also regards the growth of the Munjas and the Narengas to be the same as that of the tufted Spontaneums and the decumbent form to be on the same plan as the tufted one in principle ; but he makes only a vague distinction between a typical decumbent form (such as Local) and a typical rhizomic form (such as Glagah). The obivious difference between these and the close similarity between the modes of growth of Glagah and of his " Irrawaddy form " (which is probably identical with the Burma form grown at Coimbatore) has not been noted by him. All the same, he interprets the growth of this " Irrawaddy form " as the nearest approach to a true sympodial type.

We may now proceed to see the relation between the tufted, the prostrate and the erect forms, and inquire into the essential differences between these. The prostrate and the tufted forms agree with one another in their mode of growth. In fact, in the early stages of growth, all prostrate forms are to some extent tufted. Almost all the shoots are even from the commencement of their growth, abgeotropic and tend to grow upward as far as possible. The only difference between them is that in prostrate forms the shoots attain far greater lengths and lie across. Those growing on sandy banks are gradually buried under the sand washed ashore by the stream, and it is not impossible that these prostrate shoots are mistaken for underground stolons. Their true nature, however, is shown by the fact that their growing points like those of the tufted forms are generally sub-aerial (unless of course secondarily buried), whereas in the long, rhizomic erect forms the growing point of the " stolon " is in most cases for a long time subterranean, and turns abruptly upwards into the air once and for all, when an erect cane is to be formed. The " stolon " seems to be definitely diageotropic in response for a long time and vertical growth sets in abruptly and at a stage which is often very much delayed.

It must not be assumed, however, that the distinctions are correct to a mathematical principle. One not infrequently finds underground stolons in prostrate forms, which commence growth well below the soil and gradually curve upwards jutting out at an angle from the surface of the ground (Fig. 5). Nor is it at all uncommon to meet with buds on underground rhizomes of erect forms, which commence vertical growth almost from the very start; but the tendencies are distinctly different, and as said before, the difference is obvious in the very appearance of the resulting clumps.

(*c*) *Stability of characters.*—These differences in the extent of growth, in the strength of the culms in relation to their length and in the capacity for subterranean, radial migration are regarded by Hole as traceable to the effects of the environment. He mentions three ecological forms of Spontaneum: a xerophilous form (which probably corresponds to the Lahore form), an intermediate loam-form (Hole's), and a hygrophilous form (corresponding to E. C. C. or Local). The last mentioned form has, in his own words, a spreading habit, "to some extent at least—caused by its liability to be 'laid' by water currents, and possibly also to some extent by wind." In an earlier paragraph he says:—

> "Whatever characters are taken, however, whether the habit of growth, dimensions of the culms, width of leaves, length of spikelets, or of the callus-hair, numerous intermediates connecting the above forms can be found, and the observations in the field indicate that the characters in question vary directly according to the quality and the stability of the soil, liability to the "laying" action of wind and water, and the quantity of available moisture in the habitat."

Further, he has advanced experimental evidence, which would seem to indicate the dependence of habit and mode of growth upon environmental conditions. He finds that some of the tufted forms taken from dry sand and planted in loam have, in a single growing season, produced some wide-spreading, robust culms which resemble those of the decumbent form. This, however, has not been the case in a similar but more extensive experiment at Coimbatore. The tufted forms have been grown side by side with the prostrate forms in a clay-loam soil for the past eighteen or twenty years, well irrigated and replanted every year, still they have not shown the slightest change in their habit or mode of growth. The prostrate forms too have retained their original habit and, if anything, become more prostrate. The plants were left over to grow for a second year to see if they altered their appearance in any way; but the tufted form persisted in its original habit, and in the prostrate forms larger numbers of culms were laid across.

It is, therefore, questionable whether these habits are really subject to external influences, such as wind, water and soil. Increase or decrease of leaf-size, of waxy

deposits, alterations in the colour of cane and such other characters are known to depend largely on external influences. But habit seems to be a more deep-seated character than all these. In those cases where it was possible to compare the experimental specimens with the original plants, it has been noted that there has been no marked change in the mode of growth. In other cases, the only evidence at hand is that during the time they have been at Coimbatore, they have not altered their habit in response to the supply of water. The change of habit noted by Hole may probably be explained by the fact that all prostrate forms are at first, to a slight extent, tufted ; these plants, if removed and replanted and allowed to attain their normal adult form, naturally grow out and assume their prostrate form.

Thus so far as the present observations go, these differences are obviously inherent in them. The occurrence of intermediate forms such as Hole's "loam-form" is not at all in conflict with this assertion, for it is quite possible that there are still other modes of growth which cannot be assigned to any particular one of the three mentioned above, but which are, nevertheless, stable and inherent. Besides, it is not impossible that these tufted and prostrate forms cross with one another to a large extent in nature. Data bearing upon the case are best obtained by a study of the seedlings obtained from the various Spontaneum forms. We have at hand Barber's experience [1919] on Spontaneum seedlings and his conclusions appear to prove the two inferences just arrived at, *viz.*, that the habit characters are hereditary, and that these forms cross a great deal in nature. Barber says :—

> "In habit, the *Saccharum spontaneum* forms grown from seed vary a good deal, the young seedlings sometimes lying flat on the ground, and others growing erect and branching sparsely."

Exactly the same has been his experience in the culture of seedlings of Saretha, an Indian cane having the closest affinities to Spontaneum.

The fact that all the characters enumerated as of diagnostic importance are vegetative characters may raise the question whether vegetative characters based upon forms grown throughout by setts are reliable in distinguishing groups within a species. It may, in other words, be argued that the various specimens examined are all prolific vegetative extensions of one original bud or seed as the case may be, and are thus equivalent to a single individual plant, the length of time they have been under observation being, therefore, immaterial.

The question is easily answered. Firstly, those characters as have been seen to distinguish one plant from another are more or less of a deep-seated nature and may be regarded as the more fundamental ones among the vegetative characters and it is unlikely that they will alter when the plants are grown from seed. Several of them have been mentioned by Hackel himself. Secondly, there is the direct, though

incomplete, evidence of the behaviour of seedlings. It was mentioned earlier in the account that a selfed seedling of Dacca was obtained in the station and grown side by side with the parent; with the exception of sucrose content, no difference was observed in any of the characters between the parent and the seedling. Further, the banks of a stream in East Chitra Chavadi, near Coimbatore, are lined with Spontaneums from which the form E. C. C. was obtained. As it flowers freely in this region it may be expected that propagation takes place widely by seed. These were compared with the clumps of the same form grown in the Station, but showed no differences worth mention. Lastly some of the Spontaneums have been individually crossed with another cane, P. O. J. 2725, as the female parent and the hybrids are now being raised in the Sugarcane Breeding Station. A detailed study of these forms has not yet been made; but inspite of the fact that they all have a common mother, the P. O. J. cane, these hybrids differ largely among themselves in those characters by which their respective Spontaneum parents are broadly distinguished in the field and resemble them individually in general appearance.

We have finally to deal with the intermediate forms and forms possessing one or two features of the opposite group. Intermediates have been brought forward to indicate that there is no sharp line of demarcation between the *indicum* and the *ægyptiacum* groups. Exactly in what characters they are intermediate will be important. It has been shown that so far as the floral characters are concerned, it is impossible to make any sharp distinctions, there being gradations in some characters, the others being not concurrent. Intermediates in floral characters, which are numerous, only indicate the greater variation in, and the essential unity of, the entire group. Where intermediates occur in respect to such vegetative characters as the robustness of culms or width of leaves, it has been possible to refer them to their respective groups on the basis of the still less variable characters, such as the ligule or the "insertion" features of the lamina. It is not known whether intermediates occur in respect to the latter characters. If they do, they may have to be regarded either as of hybrid origin or as a separate category by themselves, according to the merits of the case. It may be pointed out here that the collection in the Sugarcane Breeding Station at Coimbatore is by no means exhaustive, but only representative, and it is not at all unlikely that other groups do exist, with widely differing, but constant characters.

There are two cases where the form in question has the character of the opposite group: one is Glagah, which possesses the stomatal grooves in leaves, and the other is Rellagadi, which has a habit of growth not unlike that of the East Indian forms. Such irregularities are by no means rare in the classification of natural forms. It can only be said that the main distinctions in taxonomy are always according to the majority of concurrent characters, and that, though the distinction

between the two groups is thus made clear, there is no reason why there should be no sub-groups within the main divisions.

There seems, therefore, little doubt that there are at least two, and probably three, main groups among the forms represented in Coimbatore, the East Indian and the Indian. Burma may for the present be relegated to a sub-group under the East Indian group, though in some respects it deserves to be in a group by itself co-ordinate with the other two. It is strange how this form comes to occupy a geographical area so close to the Assamese Indian forms and is yet so distinct from them. A more intimate knowledge of its natural habitats should be had before its endemism is understood. Among the Indian forms, two stand out from the rest: Lahore with its laminae reduced to the midrib and dwarfed culms, and Rellagadi with its underground stolons; but only in the case of Lahore is the difference big enough to deserve its being classified in a separate sub-group (Hackel's var. *juncifolium*) under the Indian group.

(*d*) *Taxonomic relationships*.—It remains now to determine what taxonomic rank should be assigned to the groups now distinguished. It need hardly be remarked that the spontaneum forms, diverse and widely distributed as they are, all fall together in a large number of important morphological and anatomical features, which unite them into one compact group conforming to all the generally accepted ideas of the species-concept, such for instance as have been enumerated by Babcock [1931].

1. Common structural characteristics which unite the individuals into one group, the species.

2. Characteristic features which distinguish such groups from one another.

3. Relative stability combined with more or less variability within the group.

4. Common descent of all the individuals of the group from one or more pre-existing species.

5. *Free intercourse and high (but not necessarily complete) interfertility among the individuals of the group.

6. **Absence of intercrossing and usually low fertility if not complete sterility in hybrids between different species.

7. Frequent occurrence of sub-specific groups often occupying different geographic areas, which differ more from one another in structure or interfertility or both than do the individuals composing each group.

The last feature is particularly noteworthy as it applies here with special emphasis. The occurrence of sub-specific groups and the fact that the whole group is a species are two points which mutually confirm one another.

* This point is inferred but regarded as highly probable.

** This, however, is not true of spontaneums, for they are easily crossed with the cultivated canes, and the hybrids are in most cases reported fertile.

The revision of Hackel's has shown that his subdivision holds partly true, for these forms fail to be distinguished by all the characters enumerated by him. Those of them on which reliance could be placed have been emphasised. Thus the grounds on the basis of which a subdivision has now been defined are fewer and restricted to a few but important vegetative characters. The geographical distribution is yet in support of the subdivision. It would not be in keeping with the present conceptions of taxonomic principles if emphasis be laid on the *number* of distinguishing characters, rather than on their *distinctness* and *constancy*. The last point can be decisively settled only by breeding the forms, but if the present observations indicate anything, such an experiment will throughout confirm these conclusions.

The distinction of the broad divisions being clear, one may proceed to enquire into the nature of origin of the various forms in each subdivision. It seems clear that within the main sub-species, differentiation of many diverse forms, races and varieties is being brought about by the accumulation of small variations by natural selection in diverse ecological habitats, combined with hybridization in nature. That natural selection in Indian forms has resulted in the formation of ecotypes is evident from the extensive observations of Barber [1918] and of Hole [1911]. That these ecotypes are true hereditary races which are well-differentiated and stable is also conclusively shown by the forms at Coimbatore. And finally, that hybridization in the wild forms has been taking place can be conjectured from the characters of the many intermediate types and may also be inferred from the breeding work done on allied forms. The differentiation of the groups has been wide. Even within the limits of the Indian forms we have examples of this differentiation having proceeded to a remarkable extent. There is thus little doubt that this extensive gerontogaeous species with its distribution and its adaptation has many other well-established races, among which are already a considerable number of ecotypes and sub-species, which are possibly the early indications of the formation of new sub-species and species.

Further evidence to confirm such a subdivision can be obtained from the chromosome numbers. Already some cytological evidence is available in the Spontaneums. Bremer [1925] has counted 56 chromosomes in Glagah of Java, and 40 in the Glagah of Celebes, and judging from the morphological descriptions given by him, these two Glagahs do not seem to differ from one another as much as the Indian forms differ from the Glagahs. Dutt and Subba Rao [1933] have recently given the number of the Local form to be 32. (All numbers are haploid counts). The presence of 32 chromosomes in the Local form lends a great deal of support to the separation of the Indian forms from the East Indian. It is not unlikely that all the Indian spontaneums will have the same chromosome number as the Local form.

Thus the two groups may still be ranked as sub-species, corresponding to Hackel's sub-species *indicum* and *aegyptiacum*, though they differ from one another in fewer characters than those mentioned by him. There is sufficient basis for placing Burma in a co-ordinate sub-species, especially if cytological evidence warrants it. Apart from this, the definite chromosome numbers of all the forms as well as the cytological proof (or the contradiction) of the occurrence of natural hybridization among the forms will be of great interest and are awaited.

VI. Summary.

In an attempt to group and classify the forms of *Saccharum spontaneum* grown for a number of years under uniform conditions in the Sugarcane Breeding Station at Coimbatore, it was seen that although the forms could be broadly divided into two groups corresponding to Hackel's sub-species *indicum* and *aegyptiacum*, yet these two groups are not distinguishable according to the floral characters, which show graded distinctions not corresponding to Hackel's criteria and not concurrent with the vegetative characters, though probably constant and stable. The latter are not only constant, but outstanding and unmodified by environmental conditions, and the differences between them pertaining to the laminae, the culms, etc. (regarded as directly dependant on soil, available moisture, etc.) are persistently distinctive even when the forms are grown under uniform conditions.

Importance is attached to two characters, the shape of the ligule and the narrowing of the lamina at its insertion on the sheath, the other characters being supplementary. The Indian Spontaneums are characterised by a deltoid ligule, laminae narrowed at the base, a tufted or prostrate (exceptionally erect) habit, stomatal grooves in leaves and glabrous sheaths. The East Indian forms including the Burma form on the other hand, possess a crescentic ligule, laminae not much narrowed at base, an erect habit with long underground rhizomes and hispidulous sheaths; stomatal grooves may or may not be present. The Burma form shows important differences from the East Indian forms as also does the Lahore form (Hackel's var. *juncifolium*) from the other Indian forms. These may be relegated to separate sub-groups under their respective main groups.

The bases on which the two main groups are thus separated are fewer than those on which Hackel subdivides the species into sub-species; but nevertheless, considering the constancy and distinctness of the few but important characters, the two groups may still be regarded as definite sub-species, especially if the chromosome numbers substantiate the present classification. Cytological evidence bearing upon this is desirable.

ACKNOWLEDGMENTS.

The work of investigation was carried out in the Department of Botany, Madras University, under the direction of Dr. T. Ekambaram, M.A., Ph.D., (Cantab.), to whom I am extremely grateful for valuable guidance and encouragement throughout the progress of the work. I am equally grateful to Rao Bahadur T. S. Venkatraman, B.A., I.A.S., Government Sugarcane Expert, for his kindness in suggesting the problem and providing the material and every facility for its investigation. Part of the work was carried out in the Sugarcane Station at Coimbatore. My thanks are also due to Mr. R. Thomas for his continued assistance in the fields.

VII. APPENDIX.

Key to the forms of Saccharum spontaneum *grown at Coimbatore, based on vegetative characters.*

1. Ligule deltoid; lamina narrowed at base, midrib more than half the width of the lamina at its insertion on the sheath.	Sub-species *indicum*	2
1. Ligule crescentic; lamina not narrowed at base, midrib less than half the width of the lamina at its insertion on the sheath.	Sub-species *aegyptiacum*	6
2. Laminae wiry, reduced to the midrib, culms short, densely tufted.	var. *juncifolium* Lahore.	
2. Laminae not reduced to the midrib; culms long, tufted or otherwise.		3
3. Culms ultimately prostrate		4
3. Culms erect, underground stolons present	Rellagadi.	
3. Culms tufted	Gerah Bon.	
4. Culms slender, not more than ·4-in. thick, leaves narrow, not more than ·7-in. wide.		5
4. Culms robust, over ·4-in. thick, leaves wide over ·7-in. in width.	Dacca.	
5. Leaf-sheaths purple	E.C.C.	
5. Leaf-sheaths always green	Holes and local.	
6. Internodes never outgrowing the corresponding sheaths.		7
6. Internodes generally outgrowing the corresponding sheaths.	(nov. var.) Burma.	
7. Stomatal grooves present in leaves	Glagah.	
7. Stomatal grooves absent in leaves	Sumatra.	

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STUDIES IN INDIAN PULSES

NO. 6. THE ROOT SYSTEMS OF GREEN AND BLACK GRAMS

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(With Plates LXXVII and LXXVIII)

I. Introduction

Forty different types of *mung* or green gram (*Phaseolus radiatus* Linn.), and twenty-five types of *urid* or black gram (*P. mungo* Linn. var. *roxburghii* Prain) have been evolved at the Botanical Section of the Imperial Institute of Agricultural Research, Pusa, from samples of seed originally collected from different parts of India [Bose, 1932, 1 and 2]. These types present a large amount of variation in their habit, maturity and other morphological and physiological characters, including, of course, the extent and distribution of their root systems.

Variability in the root systems of the different types of *mung* and *urid* is to be expected because the material from which these types were evolved was drawn from widely different soil and agricultural conditions. Now, a crop can only make the best use of the soil when its root system is efficiently adapted to the soil conditions and it is the duty of the plant breeder to select, from the various types of root systems available, that one which will connect the plant and the soil in the most efficient manner. In other words, the maximum results as regards yield, disease-resistance, etc., can only be obtained by a knowledge of the root systems.

It has been established by various investigators that root habit, though responsive to environmental conditions within certain limits, is primarily governed by heritable factors. This fact has very well been illustrated in India by Howard and Howard [1917] in linseed and *Lathyrus sativus* L. and by Bose and Dixit [1931] in barley. Of late a good deal of attention has been paid to the study of the root systems of different crops such as rice [Sethi, 1929], sugarcane

[Venkatraman, 1917], chillies [Ali Muhammad and Deshpande, 1929] and sesamum [Kashi Ram and Madhava Row, 1931].

II. Methods

The root habits of all the Pusa *mungs* and *urids* were studied in 1931 and were re-examined and checked in the following year. A number of plants in each type was examined and the average of all measurements of extent and distribution of the roots were recorded. The crops were always grown in the field, under normal conditions, and the method of root washing was the same as that described in the study of the root system of Indian barleys [Bose and Dixit, 1931]. The end plants of each row of all types were always discarded for these studies, on account of the influence of border effect on their root systems. Pencilled drawings were invariably made in the field of the root system of the typical plant in each individual type. The drawings were made on squared papers with lines one centimetre apart either way. It was found very convenient and easy to represent on such papers the exact position, nature of branching and the inclination of the primary and secondary roots as well as the amount of fibrous roots present.

III. General description

In 1931 the root systems of all the types of *mung* and *urid* were exposed at monthly intervals, beginning one month after the date of sowing of the crop. In the preliminary observations, however, no clear-cut differences could be found and regular differentiation could be made only at the final stage when the crop was fully mature. In the following year, therefore, only one series of observations was made and this was sufficient to support the previous year's conclusions.

In both these crops two main types of root systems could be recognized and there appeared to be a definite relation between the type of root system and the locality from which the seed originated. This agrees with the results recorded in the study of the root systems of Indian barleys [Bose and Dixit, *loc. cit.*].

The types of root system present in these two crops could be classified as follows :—

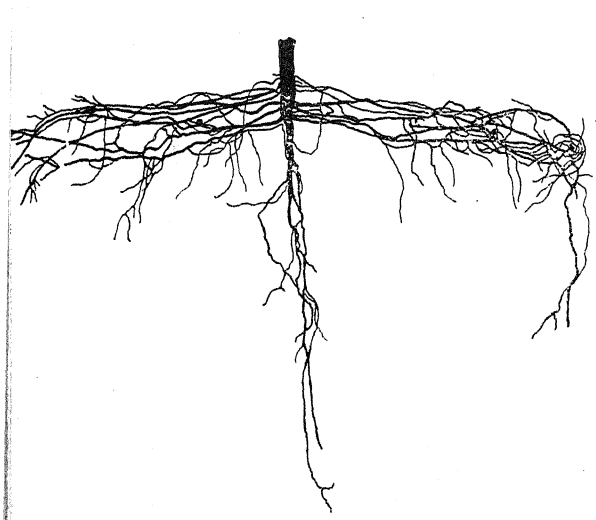
I. *Mesophytic*

and II. *Xerophytic.*

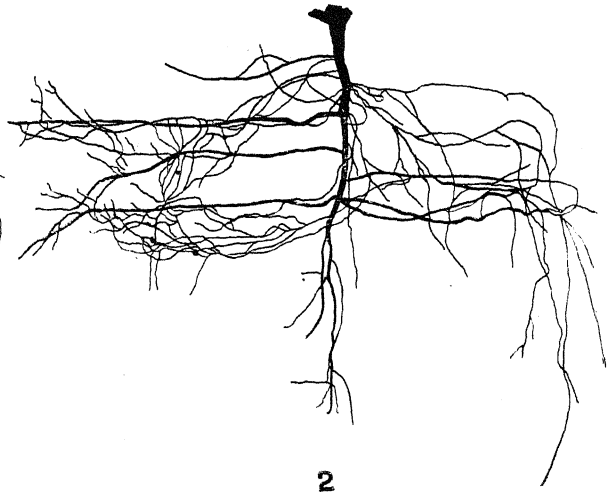
I. *Mesophytic type of root system.*—*Mung* and *urid* types with well developed and profusely branched shallow roots in the upper regions of the soil on which they depend for their moisture supply and in which the secondary roots are generally given off at right angles to the main tap root and run almost parallel to

PLATE LXXVII.

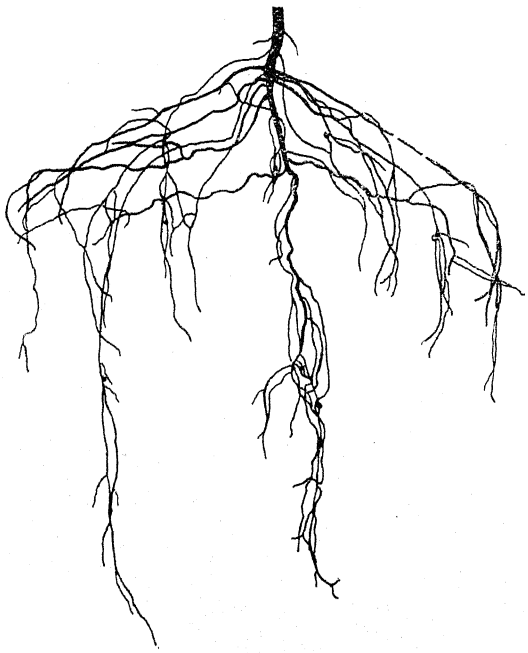
TYPES OF ROOT SYSTEMS IN *MUNG* AND *URID*.



1



2



3

4

1. Mesophytic—A. 2. Mesophytic—B. 3. Xerophytic—A. 4. Xerophytic—B.

the surface of the soil. The tap roots are fairly thick up to a depth of about three to six-and-a-half inches and this is the region where almost all the secondary roots are given off. Beyond this depth the tap roots are generally thin and hardly develop secondary roots of any importance. Sometimes a few laterals do develop in this region but they are never more than two or three inches in length.

II. *Xerophytic type of root system.*—*Mung* and *urid* types with sparsely developed, shallow root system in which the secondary roots do not develop at right angles to the main tap root as in the previous case, but they take a downward course and run obliquely down into the soil. The tap roots are fairly thick up to a depth of five to twelve inches and later become thin, and penetrate the soil up to a depth of about forty inches. Types with this system of roots entirely depend for their water supply on the lower layers of the soil and thus scarcely affect the upper layers.

These two main groups of root-types (Plate LXXVII, figs. 1 and 4) could also be sub-divided into further grades depending upon the working depths of the roots as well as on the nature of the development of the fibrous roots. Thus—

I. *Mesophytic*—

A. Secondary roots horizontal and develop abundantly up to a depth of about 4½ inches.

No. 1.—Tertiary or fibrous roots poorly developed.

No. 2.—Tertiary or fibrous roots well developed.

B. Secondary roots horizontal and develop abundantly up to a depth of about 6½ inches.

No. 3.—Tertiary or fibrous roots poorly developed.

No. 4.—Tertiary or fibrous roots well developed.

II. *Xerophytic*—

A. Secondary roots oblique and develop mostly from the upper 5 to 7 inches of the tap root.

No. 1.—Tertiary or fibrous roots poorly developed.

No. 2.—Tertiary or fibrous roots well developed.

B. Secondary roots oblique and develop mostly from 7½ to 12 inches of the tap root.

No. 3.—Tertiary or fibrous roots poorly developed.

No. 4.—Tertiary or fibrous roots well developed.

It might be mentioned that root Types 1 and 3 and 2 and 4 respectively in both the mesophytic and the xerophytic systems sometimes look more or less alike but the differences in their working depths have always been rather pronounced and hence this sub-division has had to be made.

Table I depicts the different types of root systems observed in *mung* types together with the locality from which each type originated, as well as some other morphological characters which seem to be related with the root systems.

TABLE I.

Root systems and some other characters of mung *types.*

Nature of root system	*Mung* type No.	Locality of origin	Root				Maturity of *mung* type	Plant habit
			Maximum depth	Lateral spread	Working depth	Development of fibrous roots		
Mesophytic 1	40	Murshidabad .	18·0	25·0	3·50	Poor .	Medium .	Semi-erect, leaves sparse.
,,	7	Burdwan . .	23·0	21·0	3·75	Poor .	Early .	Semi-erect, and bushy.
Mesophytic 2	9	Bhagalpur .	24·0	22·0	3·75	Fair .	Early .	Semi-erect, leafy and robust.
,,	22	Ratnagiri .	22·0	32·0	3·00	Fair .	Early .	Spreading, prolific.
,,	29	Ratnagiri .	24·5	29·5	3·75	Good .	Early .	Spreading, bushy.
,,	13	Monghyr . .	19·5	30·5	3·50	Good .	Early .	Spreading, robust.
,,	4	Sabour . .	22·5	31·5	3·75	Good .	Early .	Spreading, robust.
,,	2	Bhagalpur .	20·5	31·0	3·00	V. good .	Early .	Spreading, robust.
,,	28	Sepaya . .	17·5	21·0	3·50	V. good .	Early .	Semi-erect, prolific.
Mesophytic 3	39	Brahmanbaria .	24·0	29·0	6·50	Poor .	Late .	Spreading, bushy.
Mesophytic 4	20	Mandalay .	20·5	20·5	6·50	Good .	Medium .	Semi-erect.
Xerophytic 1	35	Mandalay .	21·5	10·5	6·00	Poor .	Medium .	Semi-erect.
,,	33	Mangalore .	19·0	10·5	6·50	Poor .	Early .	Semi-erect, poor.
,,	24	Mandalay .	20·0	12·0	6·00	Poor .	Early .	Semi-erect, prolific.
,,	14	Lyallpur . .	25·0	12·5	6·00	Poor .	Early .	Semi-erect, poor.
,,	5	Ahmedabad .	23·0	12·5	6·25	Poor .	Early .	Semi-erect, fairly bushy.
,,	12	Dohad . .	23·5	13·0	6·25	Poor .	Early .	Semi-erect, bushy.

TABLE I—*contd.*

Nature of root system	*Mung* type No.	Locality of origin	Root				Maturity of *mung* tppe	Plant habit
			Maximum depth	Lateral spread	Working depth	Development of fibrous roots		
Xerophytic 1 .	11	Nagpur . .	26·0	14·5	6·25	Poor .	Early .	Spreading, poor.
,, .	23	Schewbo North	26·0	12·5	6·75	Poor .	Early .	Semi-erect, prolific.
Xerophytic 2 .	3	Dohad . .	27·0	20·0	6·00	Fair .	Early .	Spreading, leafy.
,, .	36	Yeotmal . .	24·0	12·5	6·00	Fair .	Early .	Semi-erect.
,, .	6	Haveri . .	23·5	15·5	6·50	Fair .	Medium .	Semi-erect, bushy.
,, .	18	Ramnad . .	26·5	14·5	6·50	Fair .	Early .	Semi-erect, prolific.
,, .	21	Peshawar .	35·0	18·0	6·50	Fair .	Early .	Spreading, prolific.
,, .	25	Peshawar .	25·0	14·0	6·50	Fair .	Early .	Semi-erect, bushy.
,, .	26	Partabgarh .	26·0	19·0	6·50	Fair .	Early .	Spreading.
,, .	34	Cawnpur . .	23·0	18·0	6·50	Fair .	Early .	Spreading, poor yielder.
,, .	38	Islampur . .	25·0	10·5	6·50	Fair .	Medium .	Semi-erect.
,, .	37	Islampur . .	23·0	12·5	7·00	Fair .	Early .	Semi-erect.
,, .	27	Mandalay .	24·5	17·5	7·00	Fair .	Early .	Semi-erect, bushy.
,, .	10	Allanmyo .	23·0	14·0	7·50	Fair .	Medium .	Semi-erect, prolific.
,, .	8	Aligarh . .	18·0	13·0	7·50	Fair .	Early .	Semi-erect, bushy.
,, .	15	Gurdaspur .	21·5	12·5	5·50	Good .	Early .	Semi-erect, leafy.
,, .	1	Ramnad . .	21·5	18·5	5·75	Good .	Early .	Spreading, robust.
,, .	16	Coimbatore .	21·0	14·5	6·25	Good .	Medium .	Semi-erect, prolific.
Xerophytic 3 .	32	Bangalore. .	30·5	19·5	10·75	Poor .	Late .	Spreading.
,, .	30	Kumpta . .	22·0	17·0	12·00	Poor .	Late .	Spreading, bushy.
Xerophytic 4 .	19	Coimbatore .	27·0	18·5	8·50	V. good .	Medium .	Spreading, bushy.
,, .	31	Samalkot . .	32·0	17·5	10·75	V. good .	Late .	Spreading, prolific.
,, .	17	Coimbatore .	27·0	13·0	12·00	V. good .	Late .	Erect, bushy.

Similarly Table II given below shows the same characters for the 25 types of *urid*.

TABLE II.

Root systems and some other characters of urid *types.*

Nature of root system	*Urid* type No.	Locality of origin	Root				Maturity of *urid* type	Plant habit	Branching
			Maximum depth	Lateral spread	Working depth	Development of fibrous roots			
Mesophytic 1	16	Bhagalpur .	26·0	27·5	4·00	Poor .	Medium	Spreading	Moderate
,,	19	Burdwan .	28·5	28·0	4·00	Poor .	Medium	Spreading	Moderate
Mesophytic 2	9	Pusa . .	27·5	29·0	4·00	Fair .	Medium	Spreading	Profuse
,,	4	Pusa . .	22·5	31·0	4·50	Good .	Medium	Trailing .	Profuse
,,	6	Darbhanga .	22·5	32·0	4·50	Good .	Medium	Spreading	Profuse
,,	24	Birbhum .	29·0	22·5	4·50	Good .	Medium	Semi-erect	Profuse
Mesophytic 3	25	Gaya . .	31·0	30·0	6·50	Poor .	Late .	Spreading	Moderate
Mesophytic 4	2	Muzaffarpur .	29·0	30·5	6·25	Good .	Late .	Trailing .	Profuse
,,	12	Sabour . .	29·5	30·5	6·25	Good .	Late .	Spreading	Profuse
,,	14	Sepaya . .	24·5	29·0	6·25	V. good	Late .	Trailing .	Profuse
,,	17	Bhagalpur .	27·5	28·0	6·25	V. good	Medium	Spreading	Profuse
,,	5	Muzaffarpur .	29·0	29·0	6·50	V. good	Late .	Spreading	Profuse
,,	1	Monghyr .	31·0	34·5	6·50	V. good	Late .	Spreading	Profuse
Xerophytic 1	22	Gurdaspur .	32·0	19·0	4·50	Poor .	Early .	Spreading	Scanty
,,	11	Aligarh. .	24·5	18·5	6·00	Poor .	Medium	Spreading	Profuse
Xerophytic 2	8	Aligarh. .	28·5	20·5	5·00	Fair .	Early .	Spreading	Profuse
,,	10	Ferozepur .	30·0	19·0	5·00	V. good	Medium	Spreading	Profuse
,,	23	Tanjore. .	28·5	19·5	6·50	V. good	Medium	Trailing .	Profuse
Xerophytic 3	21	Lyallpur .	39·0	20·0	8·00	Poor .	Medium	Spreading	Profuse
,,	15	Cawnpur .	38·0	19·5	9·25	Poor .	Late .	Spreading	Profuse
Xerophytic 4	3	Cawnpur .	34·5	17·5	7·50	Good .	Medium	Spreading	Profuse
,,	7	Aligarh. .	33·0	18·5	7·50	Good .	Medium	Spreading	Profuse
,,	13	Rohtak. .	27·0	19·0	7·50	Good .	Early .	Spreading	Moderate
,,	18	Agra . .	30·0	18·5	7·50	Good .	Medium	Spreading	Profuse
,,	20	Thana . .	28·0	12·0	10·00	Good .	Medium	Semi-erect	Moderate

The relation of root habit with other characters is well apparent from the above two tables and will be discussed in some detail in the following pages. In general it may be pointed out that the maximum depth of the main tap root is greater in the xeroyhytic than in the mesophytic type of root system.

TABLE III.

Relation of the type of root system with its maximum depth in mung *and* urid *types.*

Maximum depth in inches	*Mung* (*P. radiatus* L.)		*Urid* (*P. Mungo* L. *var. Roxburghii* Prain)	
	Mesophytic roots	Xerophytic roots	Mesophytic roots	Xerophytic roots
15·1 to 17·5 . .	T. 28 . . .	...	...	...
17·6 to 20·0 . .	T. 13, T. 40 . .	T. 8, T. 24, T. 33 .	...	...
20·1 to 22·5 . .	T. 2, T. 4, T. 20, T. 22, T. 29.	T. 1, T. 15, T. 16, T. 30, T. 34, T. 35.	T. 4, T. 6 . .	...
22·6 to 25·0 . .	T. 23, T. 9, T. 39 .	T. 5, T. 6, T. 10, T. 12, T. 14, T. 25, T. 27, T. 36, T. 37, T. 38.	T. 14 . . .	T. 11.
25·1 to 27·5 . .	...	T. 3, T. 11, T. 17, T. 18, T. 19, T. 23, T. 26.	T. 9, T. 16, T. 17 .	T. 13.
27·6 to 30·0 . .	...	...	T. 2, T. 5, T. 12, T. 19, T. 24.	T. 8, T. 10, T. 18, T. 20, T. 23.
30·1 to 32·5 . .	...	T. 31, T. 32 . .	T. 1, T. 25 . .	T. 22.
32·6 to 35·0 . .	...	T. 21 . . .	...	T. 3, T. 7.
35·1 to 37·5 . .	...	...	...	...
37·6 to 40·0 . .	...	...	...	T. 15, T. 21.

It is evident from the above table that the mesophytic types in both the crops do not usually send out their main tap-roots to such depths as do the xerophytic types and also that the *urid* types in general have a deeper root system than the *mung* types.

IV. RELATION TO LOCALITY

Though root-habit is responsive to environmental conditions within certain limits, it is primarily governed by heritable factors. Nature and nurture are two important attributes on which the growth and development of all plants depends, but it may be said, without the least hesitation, that no amount of selection and

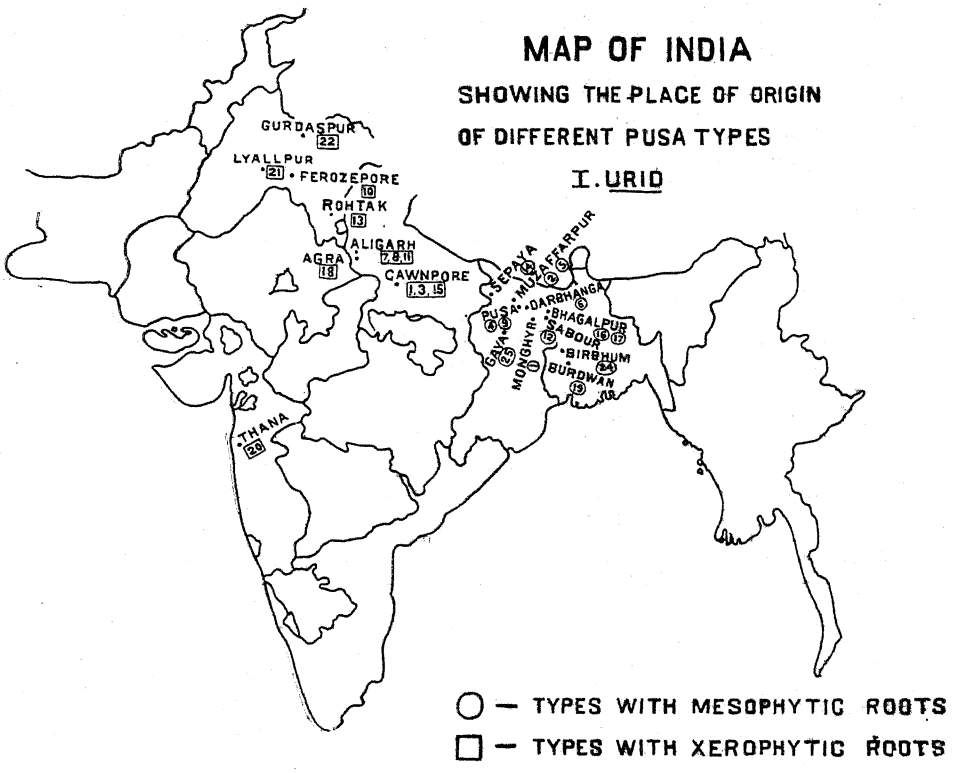
growing them under changed environmental conditions will ever tend to change the root-habit of any plant. The maps on Plate LXXVIII show the different localities from which the original seed of the various Pusa *mung* and *urid* types was drawn and also represent the nature of root system which each type possesses. It is interesting to find that all types with the mesophytic type of root-system, excepting Type 20 from Mandalay and Types 22 and 29 from Ratnagiri have originated from seeds collected from the alluvial soils of Bengal and Bihar. The other types having a xerophytic type of root system, on the other hand, have come from drier tracts of the United Provinces of Agra and Oudh, Punjab and peninsular India and some parts of Burma. It is quite possible that these three types, *viz.*, Types 20, 22, and 29 were not indigenous to the localities from which they were drawn, but had been temporarily introduced there from somewhere else.

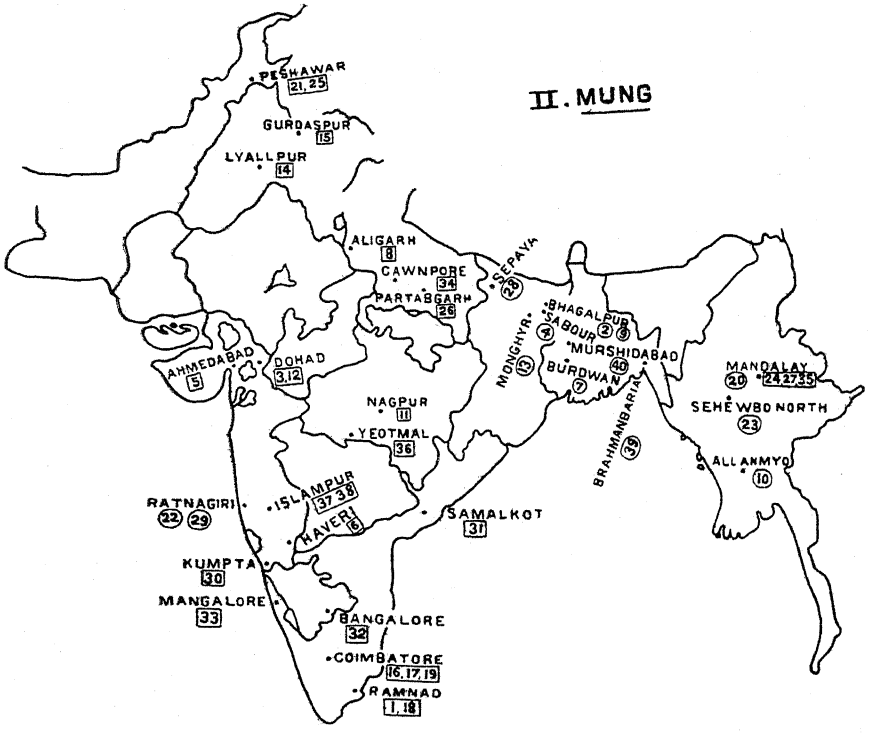
These results, it might be noted, are in conformity with those obtained in previous root studies with other crops, and the explanation regarding the distribution of the different types of root-habits is obviously centred round the two points, 1) of soil æration and (2) of the capacity of the soil to retain moisture. In the first place the anærobic condition of the lower levels of the soils in the Gangetic alluvium does not favour deep growing root systems while the presence of sufficient moisture and food materials in the upper soil layers renders the Gangetic alluvium an ideal substratum for surface-rooting types. Conversely the drier soils of peninsular India and Punjab with their deeper-seated supplies of moisture and better æration at deeper levels are fitted for the growth of types which possess xerophytic root systems.

It must be acknowledged that the types of *mung* isolated at Pusa have come from a wider range of soil and climatic conditions than those of *urid* as will be apparent from the maps in Plate LXXVIII. Samples of seed, however, of both these crops were collected from more or less the same places but most of the *urid* strains from peninsular India and some other parts, failed to grow at Pusa on account of heavy rains during the growth period of this crop which is from July to October. The *mung* crop, being grown in and round about Pusa during the dry months of March to May, did not suffer likewise and hence has remained rather more representative of the different conditions.

V. Relation to maturity

That a deep-rooting habit and a long growing season and a shallow-rooting habit and a short growing period go hand in hand has been demonstrated by many workers, *viz.*, Weaver [1926], Bose and Dixit [1931] and others. This fact is further confirmed by the present studies. Table IV shows the relation of the type of root system and its working depth with the maturity of *mung* and *urid* types.


MAP OF INDIA
SHOWING THE PLACE OF ORIGIN
OF DIFFERENT PUSA TYPES
I. URID
GURDASPUR 22
LYALLPUR 21
FEROZEPORE 10
ROHTAK 13
ALIGARH 7,8,11
AGRA 18
CAWNPORE 1,3,15
SEPAYA
MUZAFFARPUR
DARBHANGA
BHAGALPUR 16 17
SABOUR
BIRBHUM 24
BURDWAN 7 12
GAYA
MONGHYR
THANA 28
○ — TYPES WITH MESOPHYTIC ROOTS
□ — TYPES WITH XEROPHYTIC ROOTS


II. MUNG
PESHAWAR 21,25
GURDASPUR 15
LYALLPUR 14
ALIGARH 8
CAWNPORE 34
PARTABGARH 26
SEPAYA 28
BHAGALPUR 2 5
SABOUR 4
MURSHIDABAD 40
BURDWAN 7
MONGHYR 13
AHMEDABAD 5
DOHAD 3,12
NAGPUR 11
YEOTMAL 36
BRAHMANBARIA 39
MANDALAY 20 24,27,35
SEHEWBO NORTH 23
ALLANMYO 10
RATNAGIRI 22 29
ISLAMPUR 37 38
HAVERI 6
SAMALKOT 31
KUMPTA 30
MANGALORE 33
BANGALORE 32
COIMBATORE 16,17,19
RAMNAD 1,18

PI

TABLE IV.

Relation of the type of root system and their working depths with the maturity of mung *and* urid *types.*

Working depths in inches	*Mung* (*Phaseolus radiatus* Linn.)						*Urid* (*Phaseolus mungo* L. *var. Roxburghii* Prain)					
	Mesophytic roots			Xerophytic roots			Mesophyic roots			Xerophytic roots		
	Early	Medium	Late	Early	Medium	Late	Early	Medium	Late	Early	Medium	Late
2.1 to 3.0 .	T. 2, T. 22	..	..	..	..	..	..	..	..	..	..	..
3.1 to 4.0 .	T. 4, T. 7, T. 9, T. 13, T. 28, T. 29.	T. 40	..	..	..	..	..	T. 9, T. 16, T. 19.	...	..	..	..
4.1 to 5.0 .	..	..	..	..	..	..	..	T. 4, T. 6, T. 24.	..	T. 8, T. 22.	T. 10	..
5.1 to 6.0 .	T. 36	..	..	T. 1, T. 3, T. 14, T. 15, T. 24.	T. 35	..	..	..	..	..	T. 6	..
6.1 to 7.0 .	..	T. 20	T. 39	T. 5, T. 11, T. 12, T. 21, T. 18, T. 23, T. 25, T. 26, T. 27, T. 33, T. 34.	T. 6, T. 16, T. 37, T. 38.	..	..	T. 17	T. 1, T. 2, T. 5, T. 12, T. 14, T. 25.	..	T. 23	..
7.1 to 8.0 .	..	..	..	T. 8	T. 10	..	..	..	..	T. 13	T. 3, T. 7, T. 18, T. 21.	..
8.1 to 9.0 .	..	..	..	..	T. 19	..	..	..	..	..	..	..
9.1 to 10.0 .	..	..	..	..	..	..	..	..	..	..	T. 20	T. 15
10.1 to 11.0.	..	..	..	..	..	T. 31, T. 32.	..	..	..	..	..	..
11.1 to 12.0.	..	..	..	..	..	T. 17, T. 30.	..	..	..	..	..	..

It will be seen from the above table that all early maturing forms both of *mung* and *urid* have a shallow working depth, while all late maturing types have a deeper working depth of the roots and also that the types which are medium in maturity are more or less intermediate as regards this character. Further it is also evident that the xerophytic types have a distinctly deeper working depth of their roots than the mesophytic types.

VI. Relation to habit

There is also a definite relation between the lateral spread of the roots and the general habit of the upper parts of the plant. From the table given below it may be noted that the lateral spread of secondary roots is much greater in the mesophytic than in the xerophytic types of root systems and that types with a spreading habit of the plant have also a greater lateral spread of the roots than types with the erect and semi-erect habit of the plant.

Table V.

Relation of type of root system and the lateral spread of the secondary roots with the habit of plant in mung *and* urid.

Lateral spread of secondary roots in inches	*Mung* (*Phaseolus radiatus* Linn.)				*Urid* (*Phaseolus mungo* L. *var. Roxburghii* Prain)			
	Mesophytic roots		Xerophytic roots		Mesophytic roots		Xerophytic roots	
	Spreading	Erect or semi-erect	Spreading	Erect or semi-erect	Spreading	Erect or semi-erect	Spreading	Erect or semi-erect
10.1 to 12.5 .	..	..	..	T. 5, T. 14, T. 15, T. 17, T. 23, T. 24, T. 33, T. 35, T. 36, T. 37, T. 38	..	..	..	T. 20
12.6 to 15.0 .	..	..	T. 11	T. 8, T. 10, T. 12, T. 16, T. 18, T. 25.	..	..	..	..
15.1 to 17.5 .	..	..	T. 30, T. 31	T. 6, T. 27	..	..	T. 3	..
17.6 to 20.0 .	..	..	T. 1, T. 3, T. 19, T. 21, T. 26, T. 32, T. 34.	..	..	..	T. 7, T. 10, T. 11, T. 13, T. 15, T. 18, T. 21, T. 22, T. 23.	..
20.1 to 22.5 .	..	T. 7, T. 9, T. 20, T. 28.	..	..	..	T. 24	T. 8	..
22.6 to 25.0 .	..	T. 40.	..	..	..	..	..	..
25.1 to 27.5 .	..	..	..	..	T. 16	..	..	..
27.6 to 30.0 .	T. 29, T. 39.	..	..	..	T. 5, T. 9, T. 14, T. 17, T. 19.	..	..	..
30·1 to 32.5 .	T. 2, T. 4, T. 13, T. 22.	..	..	..	T. 2, T. 4, T. 12, T. 25.	..	..	..
32.6 to 35.0 .	..	..	..	..	T. 1, T. 6	..	..	..

It may be generalized that in *mung* and *urid* the range of the lateral spread of secondary roots is somewhat as follows :—

Root habit	Shoot habit	Range of lateral spread in inches	
		Mung	*Urid*
Mesophytic . .	Erect or semi-erect . .	20·1 to 25·0	20·1 to 22·5
„ . .	Spreading	27·6 to 32·5	25·1 to 35·0
Xerophytic . .	Erect or semi-erect . .	10·1 to 17·5	10·1 to 12·5
„ . .	Spreading	12·6 to 20·0	15·1 to 22·5

These facts thus confirm the general rule that the root habit of a plant is more or less the mirror image of the shoot habit. This information regarding the habit and spread of root systems in the various types of *mung* and *urid* can be utilized most advantageously as a guide in the spacing that should be given to each type under field conditions. Obviously the spacing will vary directly with the extent of the lateral spread of secondary roots.

VII. Summary and conclusions

The root systems of forty types of *mung* or green gram (*Phaseolus radiatus* Linn.) and twenty-five types of *urid* or black gram (*Phaseolus mungo* L. *var. Roxburghii* Prain) isolated at Pusa have been studied and their relationship with some other characters determined. The popular method of root washing with the help of a knapsack sprayer was used.

In both the crops the following classes of root systems were observed :—

I. Mesophytic—

A. Secondary roots horizontal and develop abundantly up to a depth of about 4½ inches.

No. 1.—Tertiary or fibrous roots poorly developed.
No. 2.—Tertiary or fibrous roots well developed.

B. Secondary roots horizontal and develop abundantly up to a depth of about 6½ inches.

No. 3.—Tertiary or fibrous roots poorly developed.
No. 4.—Tertiary or fibrous roots well developed.

II. Xerophytic—

A. Secondary roots oblique and develop mostly from the upper 5 to 7 inches of the tap root.

No. 1.—Tertiary or fibrous roots poorly developed.
No. 2.—Tertiary or fibrous roots well developed.

B. Secondary roots oblique and develop mostly from $7\frac{1}{2}$ to 12 inches of the tap root.

No. 3.—Tertiary or fibrous roots poorly developed.
No. 4.—Tertiary or fibrous roots well developed.

It was found that the maximum depth of the main tap root was greater in the xerophytic than in the mesophytic types of both *mung* and *urid*, but that *urid* generally had a distinctly deeper root penetration than *mung*.

Types of *mung* and *urid* coming from the alluviums of Bengal and Bihar invariably had a mesophytic type of root system, whereas types which originated from seed collected from drier localities of the United Provinces of Agra and Oudh, Punjab, North-West Frontier Province and Peninsular India as well as most of the types from Burma, all possessed the xerophytic type of root system.

All early maturing forms of *mung* and *urid* had a shallow working depth of their roots while all late maturing types had a deeper working depth. Types which were medium in maturity were also intermediate as regards this character.

The lateral spread of secondary roots was much greater in the mesophytic than in the xerophytic types of root systems. Types with a spreading habit of the plants had a greater lateral spread of the roots than types with the erect or semi-erect plant habit.

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PHOTO-NITRIFICATION OF SOME COMPOUNDS.

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A number of chemical reactions involved in the oxidation of ammonia are known since long. Ammonia, for instance, can be oxidised in the presence of copper oxyhydrate and ferric hydrate. Ammonia can also be oxidised by ultra violet rays and by interaction with ozone and hydrogen peroxide. These chemical changes are, however, of no importance in the soil. Nitrification in soils has hitherto been universally believed to be exclusively biological in nature [Waksman, 1927].

It has recently been shown by Rao and Dhar [1931] that ammonia and its salts of mineral acids are rapidly oxidised photo-chemically to nitrite in the presence of a sensitizer such as, zinc oxide. Since certain facts were not explainable on biological basis, they suggested that photo-chemical nitrification was also going on side by side with the microbiological process.

Zo Bell [1933] found measurable increase in the nitrite and nitrate content of sea water, when it was added to ammonium salts in the presence of sunlight.

Palit and Dhar [1926, 1930] carried out a systematic investigation of the photo-chemical oxidation of some of the compounds (urea, hippuric acid and glycocoll) included in this study. They, however, studied this from the view point of oxidations in the living cell, and did not observe the ultimate fate of nitrogen during the process.

It is a well known fact that amino-acids in aqueous solution are oxidised to carbon dioxide and ammonia in the presence of blood charcoal.

The work reported in this paper was undertaken with a view to find out if nitrogenous organic compounds can also be oxidised to nitrite photo-chemically and also if ordinary field soil can act as a photo-sensitizer.

Methods of analysis.

(*i*) *Estimation of nitrous nitrogen.*—Griess Illosvay method.

(*ii*) *Estimation of nitric nitrogen.*—Phenol-disulphonic acid method.

In certain cases interfering colours were produced, due to the presence of organic matter, but the results were not materially affected.

Experimental.

One per cent. solutions of urea, glycocoll and acetamide were exposed to sunlight in 300-c. c. Erlenmeyer flasks. Zinc oxide was used as a sensitizer in the quantities of two grams in each case.

Preliminary observations were made after three days' exposure to sun in December and it was found that appreciable amount of nitrite was formed in all cases. This led to the study of some other nitrogenous compounds. Eleven different organic compounds were tried. One per cent. solutions of mono-ethyl amine, diethyl amine and aniline containing 105, 140, 155 mgms. nitrogen per 100 c. c. solution respectively, were prepared and the other compounds were used in quantities equivalent to 10 mgms. of nitrogen per 100 c. c. solution. Fifty c. c. of these solutions were placed in twenty-two 300-c. c. Erlenmeyer flasks which were divided into two series of eleven flasks each. Two grams of zinc oxide were added to the flasks in one series and five grams of normal soil (clay loam, sufficient to cover the bottom of the flasks) were added to those of the other. A third series of flasks was prepared to serve as control to which no sensitizer was added. With each of the first two series a flask containing 50 c. c. of distilled water with the requisite amount of sensitizer (zinc oxide and normal soil) was placed. All the solutions were sterilized. Mono-ethyl amine, diethyl amine and aniline were added with a sterile pipette in 50 c. c. of sterilized water in the flasks with and without sensitizer. These were all exposed to the sun over roof with such an arrangement that the maximum amount of it was availed of during the day. These were shaken occasionally, and the catalyst was kept well spread at the bottom.

Results obtained.

The contents of the flasks were analysed after 7 and 14 days' exposure. To detect bacterial contamination, one c. c. of the solution in each flask was plated on nutrient agar at the end of 14 days, and all the flasks (except two of uric acid and dicyan-diamide with soil as sensitizer) were found sterile. The results obtained are recorded in Tables I and II.

TABLE I.

Showing photo-oxidation of the solutions with zinc oxide as catalyst.

Mgms. of nitrogen per 100 c. c. solution.

Serial No.	Compound	Seven days nitrous N	Fourteen days		
			Nitrous N	Nitric N	Percentage oxidised
1	Uric Acid	0·052	0·105	0·18	1·35
2	Hippuric Acid	0·037	0·078	0·20	1·28
3	Acetamide	0·071	0·233	0·15	2·33
4	Glycocoll	0·480	0·480	0·32	6·50
5	Urea	0·015	0·018	1·48	13·48
6	Ammonium acetate	0·324	0·551	0·13	6·31
7	Ammonium oxalate	0·324	0·146	1·02	10·16
8	Dicyan-diamide	0·014	0·017	0·78	6·47
9	Mono-ethyl amine	1·372	1·701	1·53	2·93
10	Diethyl amine	0·713	1·044	0·78	1·40
11	Aniline	0·037	0·041	..	..
12	Dist. water	*Nil*	*Nil*	0·15	..

TABLE II.

Showing photo-oxidation with soil as a catalyst.

Serial No.	Compounds	Seven days		Fourteen days		
		Nitrous N	Excess over control	Nitrous N	Excess over control	Percentage oxidised
1	Uric acid	0·016	0·000	0·019	0·003	0·03
2	Hippuric acid	0·031	0·015	0·071	0·055	0·55
3	Acetamide	0·016	0·000	0·018	0·002	0·02
4	Glycocoll.	0·018	0·002	0·018	0·002	0·02
5	Urea	0·017	0·001	0·058	0·042	0·42
6	Ammonium acetate	0·024	0·008	0·142	0·126	1·26
7	Ammonium oxalate	0·016	0·000	0·016	0·000	0·00
8	Dicyan-diamide	0·019	0·003	0·021	0·005	0·05
9	Mono-ethyl amine	0·259	0·243	0·207	0·191	0·18
10	Diethyl amine	0·211	0·195	0·233	0·217	0·16
11	Aniline	0·415	0·399	0·285	0·269	0·17
12	Dist. water (control)	0·016	..	0·016	..	..

These indicate that all the compounds tried were oxidised when exposed to the sun in the presence of zinc oxide, maximum oxidation being in case of urea and

mono-ethyl amine out of the other three (liquids). In this series, all the solutions except those of the amines and ammonium salts were tested with Nessler's solution and were found to contain ammonium radical.

It is evident from the results given in Table II that the soil acts as a very weak sensitizer and that even only in the case of a few compounds, hippuric acid, urea, ammonium acetate, mono-ethyl amine, diethyl amine and aniline. It is interesting to note that exidation in the case of aniline with soil as sensitizer was more vigorous than with zinc oxide. This was repeated and confirmed. No nitrite, however, was formed in aniline solution with soil when kept in the dark showing thereby that the oxidation is purely a photo-chemical one.

In the case of solutions without sensitizer only mono-ethyl amine showed appreciable nitrous nitrogen (0·013 mgm. per 100 c.c. solution) when analysed after a week. The rest of the solutions either showed a faint trace or nil.

Oxidation of ammonium sulphate solution with different soils as sensitizer.

In order to see if different soils act as photo-catalysts in ammonium sulphate solution, six soils were selected ranging from one per cent. to 33 per cent clay. From the sandy soil (containing one per cent. clay) the sand was separated by washing off the fine fraction, and from the clay soil (containing 33 per cent. clay) fine fraction was separated by allowing the soil emulsion to settle for about five minutes.

Fifty c. c. of ammonium sulphate solution (10 mgm. of nitrogen per 100 c.c. solution) and 50 c.c. of distilled water were put in duplicates in 300-c.c. Erlenmeyer flasks respectively. Five grams of soil were added to each of the flasks and sterilized. These were exposed to the sun over roof and analysed for nitrous and nitric nitrogen after a fortnight. The results obtained are given in Table III.

TABLE III.

Showing the inactivity of different types of soils as photo-sensitizers on ammonium sulphate.

Soil No.	Percentage clay in the soil	$(NH_4)_2 SO_4$ solution		Distilled water	
		Nitrous N	Nitric N	Nitrous N	Nitric N
1	Sand	Trace	Nil	Trace	Nil
2	1	,,	,,	,,	,,
3	6	,,	,,	,,	,,
4	11	,,	,,	,,	,,
5	18	,,	,,	,,	trace
6	23	,,	,,	,,	,,
7	33	,,	,,	,,	,,
8	Fine fractions	,,	,,	,,	,,

These show that none of the soils acted as photo-catalyst on the oxidation of ammonium sulphate solution in a fortnight.

Animal charcoal as a catalyst.

Action of animal charcoal was also compared with that of zinc oxide as a catalyst and it was found after a week that oxidation of ammonium sulphate solution (10 mgms. of nitrogen per 100 c.c. solution) was more vigorous under the influence of animal charcoal as sensitizer than zinc oxide as per Table IV.

Table IV.

Showing the catalytic action of zinc oxide and animal charcoal on the oxidation of ammonium sulphate solution.

Sensitizer	Nitrous nitrogen	Percentage oxidised
1. Zinc oxide (*a*)	0·026	0·26
(*b*)	0·026	0·26
2. Animal charcoal (*a*)	0·168	1·68
(*b*)	0·175	1·75

Ammonium sulphate solutions treated with wood charcoal as sensitizer showed only a faint trace of nitrite.

Discussion of results.

From the results given above it will be observed that some of the solutions were low in nitrite content at the end of 2nd week. This appears to be due to the oxidation of nitrite to nitrate.

The oxidation of sodium nitrite solution was tried with and without a catalyst.

Table V.

Showing the oxidation of sodium nitrite mgms. nitrogen per 100 c.c. solution.

Serial No.	Treatment	1st week		2nd week		Percentage oxidised
		Nitrous N	Nitric N	Nitrous N	Nitric N	
1	Without ZnO	11·016	1·3	10·368	1·3	0·0
2	Do.	11·016	1·4	11·016	1·3	0·0
3	With ZnO	5·832	6·0	4·536	7·0	51·7
4	Do.	5·832	5·8	4·860	6·8	49·9

The results given in Table V show that sodium nitrite was oxidised to nitrate in the presence of a sensitizer, but the oxidation did not take place in its absence. This conforms to the findings of Palit and Dhar [1928], who observed no nitrate formation from sodium nitrite when air was bubbled through the solution for 5½ hours in the sun.

Aqueous solution of aniline readily underwent decomposition in the sun and was changed to a dark brown turbid solution, but when analysed after a week it did not show any nitrite. However, in the presence of catalysts pronounced oxidation took place. It was rather surprising to note that soil which acted as a very feeble catalyst surpassed zinc oxide in oxidising aniline. This might be due to the further decomposition of the dark brown product by the soil complex into easily oxidisable compounds.

Soil has shown a very feeble catalytic action, and that only in the case of some organic compounds. A number of field soils tried as catalysts did not show any action in the case of ammonium sulphate. If 50 c.c. of dilute Omliansky solution, containing about 10 mgms. of nitrogen as ammonium sulphate per 100 c.c. solution, be inoculated with a small quantity of soil and incubated at 30°C., about 5 mgms. of nitrogen was nitrified in a fortnight, while the photo-catalytic action of the soil in the same period was not appreciable at all. From this we might speculate that photo-chemical nitrification in soils is of little significance when compared with the microbial process. However, this needs further examination with different types of soils.

Soils used in these experiments were sterilized in the autoclave. It is possible that the catalytic property of soils might have been adversely affected by heat, since soils are changed both physically and chemically by autoclaving.

The presence of ammonium radical in the nitrifying solutions indicates that organic compounds were first oxidised to ammonium compounds which were subsequently changed to nitrite.

Animal charcoal was found to be a more effective photo-catalyst than zinc oxide, while wood charcoal showed but a very feeble action on the oxidation of ammonium sulphate.

Conclusions.

1. Organic compounds can be nitrified photo-chemically.

2. Field soil acted as a feeble catalyst in the oxidation of some of the organic compounds. It, however, did not show any catalytic action* on the oxidation of ammonium sulphate.

3. Sodium nitrite was oxidised to nitrate in the presence of zinc oxide.

4. Animal charcoal as a catalyst was stronger than zinc oxide.

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Note (added on 2nd August 1933.)

* Since this article was submitted for publication (April 12, 1933) our attention has been drawn to a paper on "Photo-nitrification in Soil" by Dhar, Bhattacharya and Biswas in '*Soil Science*' Vol. XXXV, pp. 281-284 (April 1933) in which the authors have come to the conclusion that the process of nitrification in soil in the tropics is more of a photo-chemical than of bacterial origin. The conclusion, however, requires confirmation under a set of different conditions. The work is in progress.

FURTHER EXPERIMENTS ON THE ROOT-GALL NEMATODE, *HETERODERA MARIONI* (CORNU) GOODEY IN SOUTH INDIA.

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(Received for publication on 1st May 1933)

(With Plates LXXIX and LXXX)

INTRODUCTION.

The root-inhabiting nematode, *Heterodera marioni* (Cornu) Goodey has been known to be the cause of one of the most destructive plant diseases in South India for the past several years. A brief history of the disease with an account of the biology of the parasite, its range of host plants and distribution and some measures of control tried in South India have been described by the writer in previous papers [Krishna Ayyar, 1926, 1933]. The purpose of the present paper is to present the results of more recent studies of this nematode, particularly in regard to host preferences, depth-distribution and some methods of control under the conditions obtaining in South India.

HOST PREFERENCES.

In the practical control of this pest either by crop rotation or by cultural methods an accurate knowledge of the comparative susceptibility or resistance of different kinds of agricultural crops will be of utmost value, and these factors have already been investigated and recorded [Krishna Ayyar, 1933]. Another factor of great economic significance which requires elucidation is the range of potential host plants regarding which no data are available in this country. The experiment described below, which throws some light on this aspect of the problem, was started towards the end of 1931 and continued until the middle of 1932.

METHODS OF STUDY.

The experiment consisted in growing a number of plants in soil heavily infected with *Heterodera*. The soil used was taken from a plot in the local insectary compound attached to the Agricultural College which was noted to be highly infective. From around tomato plants badly diseased and heavily galled by nematodes, soil was removed and loosened, and a small quantity of ordinary sand incorporated. Small earthen pots were filled with this infective soil. About twenty-five seeds of each of the following crops were sown separately in each pot on 5th December 1931. The pots were carefully watered and kept under the same condi-

tions of moisture, air, light and temperature. The climatic and other conditions being the same for all the different varieties, any variation shown in the nature of the attack should form a sound basis for an empirical study of the comparative susceptibility or immunity of the plants concerned. A few plants from each pot were carefully uprooted and the roots examined at intervals. The varying degrees of infection and resistance as shown by the abundance or absence of root-galls are indicated in the following table. The plants are arranged in descending order of severity of infection.

Relative susceptibility or resistance of plants.

Scientific name of plant	Popular name	Nature of infection and remarks
Hibiscus esculentus	Bhindi	All roots most severely galled.
Hibiscus cannabinus	Gogu	Do.
Phaseolus radiatus	Green gram	Severe infection.
Cicer arietinum	Bengal gram	Severely knotted.
Phaseolus mungo	Black gram	Do.
Dolichos biflorus	Horse gram	Do.
Medicago sativa	Lucerne	Abundantly infected.
Cyamopsis tetragonolobus	Cluster bean	Do.
Vigna catjang	Cowpea	Mild infection.
Cucurbita maxima	Pumpkin	Do.
Arachis hypogaea	Groundnuts	Do.
Sesbania aegyptiaca	*Dhaincha*	Do.
Capsicum annuum	Chillie	Slight infection.
Dolichos Lablab	Lablab	Do.
Linum usitatissimum	Linseed	Do.
Sesamum indicum	Gingelly	Do.
Trigonella faenum graecum	Venthayam	Do.
Eleusine coracana	*Ragi*	Very slight infection only in one case at root-tip.
Coriandrum sativum	Coriander	Doubtful infection.
Gossypium hirsutum	Cotton (Cambodia)	No infection.
Ricinus communis	Castor	Do.

Scientific name of plant	Popular name	Nature of infection and remarks
Cajanus indicus	Red gram	No infection.
Carthamus tinctorius	Safflower	Do.
Zea Mays	Maize	Do.
Crotolaria juncea	Sunnhemp	Do.
Panicum miliaceum	Panivaragu	Do.
Panicum miliare	Sanai	Do.
Guizotia abyssinica	Niger seed	Do.
Paspalum scrobiculatum	Varagu	Do.
Oryza sativa	Paddy	Do.
Pennisetum typhoideum	Cumbu	Do.
Setaria italica	Thenai	Do.
Andropogon Sorghum	*Cholam*	Do.

Discussion of results.

It is interesting to note that some plants are most susceptible like *bhindi*, Bengal gram, etc., and a few are completely immune under the conditions provided. Among the susceptible groups some are more severely attacked than others. The external symptoms were not uniform. Some attacked plants were stunted and unhealthy while others remained apparently uninjured. Those crops that exhibited severe infection perhaps form the natural hosts in the field. Further among the suceptibles, species of plants belonging to widely different families are noticed, while on the other hand some closely related varieties show striking differences in resistance. Among those noted to be resistant species, there are a few that have been recorded as natural hosts elsewhere. The susceptibility or resistance of a plant may not be merely dependent upon inherent differences in the plants themselves but also on factors such as soil, climate or the host history of the parasite. Whether the plants grouped together as immune will lose their power of resistance under different conditions or after an extended period of cultivation in infected soil are points for further investigation. Another point that requires elucidation is the factor or factors that render some plants unsuitable for nematode infestation. It is clear, however, that Nature in its infinite bounty has provided in these resistant varieties some means of control of the disease,

The value of such experiments, as those described, lies in the fact that such data will be of practical importance in developing varieties which can withstand the unfavourable conditions induced by eelworms. Those that exhibited any tendency to infection are to be carefully eschewed from infected soil as they have betrayed themselves to be potential hosts.

Depth distribution of *Heterodera* in South Indian soils.

In the application of chemicals for soil disinfection against *Heterodera* a knowledge of the nematode content of the soil at varying depths is indispensable. The extent to which the chemical has to penetrate into the soil and the quantity of material required are directly dependent upon the depth at which the nematodes occur and their distribution in the different layers of the soil. Lack of accurate knowledge of this factor has resulted in the failure of many experiments in contol and has also often given rise to misleading conclusions. A pioneer attempt in estimating the nematode population of soil was made by Godfrey [1924] for Florida soils. But this problem requires to be worked out for each country, locality and type of soil. With a view, therefore, to determine the distribution of *Heterodera* in South Indian soils the following preliminary experiment was conducted.

Experimental procedure.

Soil samples in sufficient quantities from different depths from the heavily infected insectary compound were removed in earthen pots and susceptible plants were sown in these under uniform conditions. The percentage of infected roots to the total number was ascertained and the same was considered roughly to form an index to the numerical strength of the population of the particular layer. In the present experiment six samples of soil from six different layers were taken in the following order in duplicates :—

I	sample	Surface level		to 6	inches	depth.
II	,,	6	inches	to 12	inches	depth.
III	,,	12	,,	to 18	,,	,,
IV	,,	18	,,	to 24	,,	,,
V	,,	24	,,	to 30	,,	,,
VI	,,	30	,,	to 36	,,	,,

The soil from each layer was carefully removed without any admixture of soil from other layers, and placed in two small flower pots. Nearly a dozen *bhindi* seeds (the most susceptible of crops in South India) were sown in each of the pots on 4th March 1932. After the lapse of about a month a few plants from each pot were carefully lifted and counts of roots made as follows. Such readings

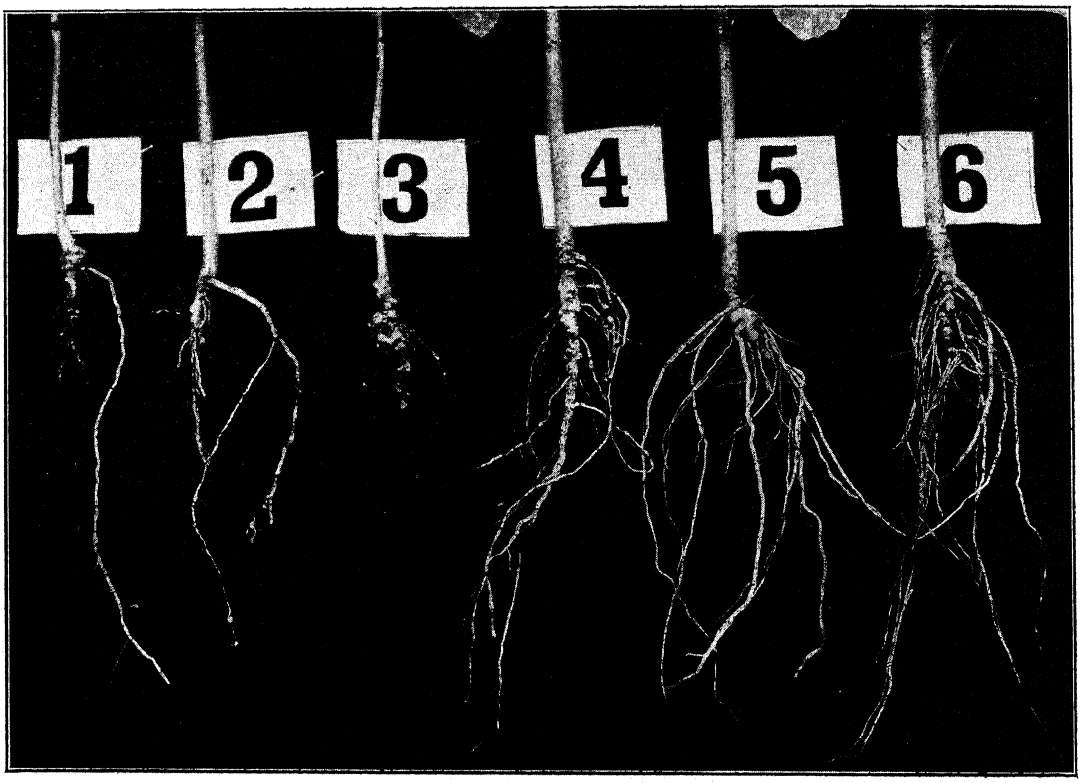
were recorded for a number of plants at intervals and some photographs of the plants were taken. The percentages of infected roots to the total number were seen to afford a satisfactory and comparative numerical estimate of the population in different layers.

Readings and results.

In every case counts of the total number of large and small roots and the number of affected ones were made and the percentage of the latter calculated. As far as possible the numbers of galls in each root as well as their sizes have also been taken into consideration. From eye impressions and actual calculations the former readings were seen to be more reliable.

Pot number	Different layers (inches)	Number of large and small roots	Number of roots with galls	Percentage	Date of examination	Nature of affection and remarks
I	Surface to 6 inches	11	6	55	6—4—32	♀ well formed, eggs not developed, galls less than 1 mm. in size.
II	6—12	16	15	94	,,	Well affected, galls 1 mm.—2½ mm.—a few eggs.
III	12—18	18	18	100	,,	Most affected, large galls, 2½ mm. in size, sickly.
IV	18—24	20	4	20	,,	Plants more or less healthy, small galls.
V	24—30	18	1	6	,,	Doubtful attack, healthy.
VI	30—36	20	Nil	Nil	,,	Healthy, free from attack.
I	Surface to 6 inches	31	28	90	8—4—32	Eggs in numbers and larvae.
II	6—12	28	24	86	,,	Galls larger in size.
III	12—18	30	29	97	,,	Eggs and larvae in plenty.
IV	18—24	29	6	21	,,	Small galls.
V	24—30	25	1	4	,,	Very small.
VI	30—36	22	Nil	Nil	,,	No infection.

Fig. 1.—(*1*) Plant from I layer of 6 in.—heavily galled.
(*2*) Plant from II layer of 6 in.—heavily galled.
(*3*) Plant from III layer of 6 in.—most heavily knotted.
(*4*) Plant from IV layer of 6 in.—slight infection.
(*5*) Plant from V layer of 6 in.—very mild, only one gall.
(*6*) Plant from VI layer of 6 in.—no infection at all.



2.—(*1*) Plant from I layer of 6 in.—heavily knotted.
(*4*) Plant from IV layer of 6 in.—slight infection.

Pot number	Different layers (inches)	Number of large and small roots	Number of roots with galls	Percentage	Date of examination	Nature of affection and remarks
I	Surface to 6 inches	116	17	15	21—5—32	
II	6—12	57	12	21	,,	
III	12—18	29	21	72	,,	Galls larger in size.
IV	18—24	74	4	5	,,	
V	24—30	44	1	2	,,	
VI	30—36	46	1	2	,,	Almost free from infection, only one minute gall.
I	Surface to 6 inches	25	25	100	10—8—32	
II	6—12	18	18	100	,,	
III	12—18	13	11	85	,,	Large galls, 3 mm.—8 mm. in size.
IV	18—24	9	5	56	,,	
V	24—30	17	1	6	,,	Very minute gall.
VI	30—36	16	Doubtful	?	,,	One small root doubtfully attacked.

The averages of four readings at different dates are as below :—

Soil layer.	*Percentage of infection.*
I layer of 6 in.	65
II ,,	75
III ,,	88
IV ,,	25
V ,,	4
VI ,,	0·5

The experiment though manifestly imperfect yields results which are somewhere near truth. The percentages quoted above form a reliable basis for comparison and approximately represent a numerical estimate of the population at varying depths. But the results are best appreciated by a reference to Plate LXXIX. The region of maximum infestation is between one foot to a foot and a half, and that of minimum is below a depth of two and a half feet. From the heavily infective soil of the surface there is a gradual increase in nematode population up to a depth of a foot and a half. There is a sudden drop in nematode contents from this layer to a depth of two feet and a half after which layer nematodes are extremely

rare. Seasonal variations, however, may slightly alter the distribution but the above estimates provide reliable data on the nematode contents at various depths in Coimbatore soils which should, in a way, be applicable to South Indian Soils.

Control measures.

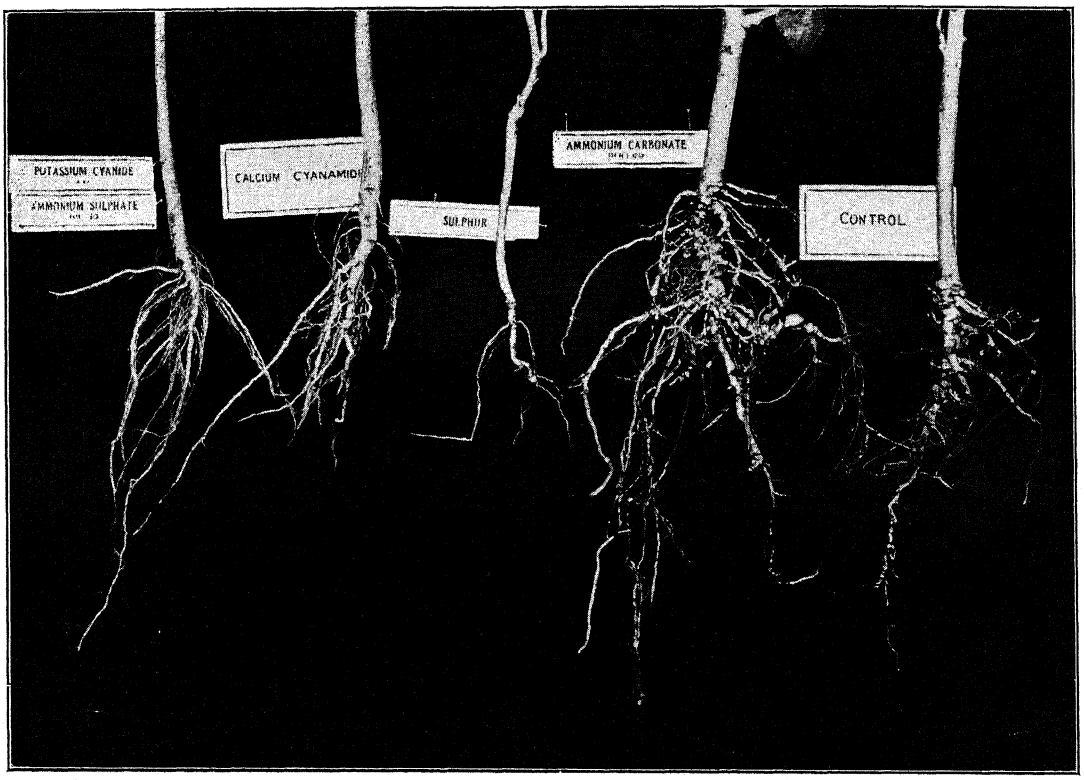
Sterilization of soils by chemicals.

The efficacy of a few substances in reducing the nematode populations in the soil under South Indian conditions has been investigated [Krishna Ayyar, 1933]. In the following paragraphs tests with a few other chemicals are described. The experiments were conducted in small earthen pots filled with heavily infected soil. Leaving two control pots the soil samples in other pots were treated with one of the chemicals enumerated below in the proportion indicated against each. In the treatments with potassium cyanide *plus* ammonium sulphate, calcium cyanamide and calcium cyanide an interval of five weeks was allowed before sowing of test crop in order to avoid their burning effects. All the pots were sown each with a dozen *bhindi* seeds (the best test crop in South India) on 4th March 1932. The test with calcium cyanide was done separately at a different date. A few plants were uprooted from each of the pots including the control for the first time on 6th April 1932 just after a month. Thereafter a periodical examination of plants was conducted for a period of three months. The results are expressed in the following table :—

Relative efficacy of chemicals.

Substances tested	Rate of application	Degree of infection in plants
Potassium cyanide *plus* ammonium sulphate.	800 lbs. and 1,000 lbs. per acre.	No infection at all. Not one root galled. All free and normal.
Calcium cyanamide .	1,000 lb. per acre . .	Very slightly affected having a few small galls one mm. in size.
Sulphur . . .	800 lb. per acre . .	Badly knotted roots, galls large, numerous, no relief afforded.
Ammonium carbonate .	800 lb. per acre .	Very severely galled but plants had very vigorous growth ; no better than controls.
Controls . . .	..	Very heavy infestation.
Calcium cyanide . .	800 lb. per acre . .	Moderate infection, better than controls, slight relief.

EFFICACY TESTS



Potassium cyanide and ammonium sulphate . . .	No trace of root-galls.
Calcium cyanamide.	Very few galls.
Sulphur, Ammonium carbonate, Control	All heavily galled. No appreciable relief.

Results.

This investigation, comparatively simple in itself, yields some useful results which justify definite conclusions. Plate LXXX reveals clearly the extent of efficacy of the different treatments. The application of calcium cyanide in the proportion tried does not appear to afford much relief under South Indian conditions. The sulphur and ammonium carbonate treatments have shown no marked improvement from controls except that the latter, as would be expected, stimulated plant growth. Hence their use under these conditions will not be productive of any good results as soil disinfectants against *Heterodera*. The efficacy of potassium cyanide *plus* ammonium sulphate (Plate LXXX) is amply proved by the total absence of any trace of galls on roots for a period of over three months after application. This is in complete conformity with the findings of other investigators. This method can therefore be recommended under South Indian conditions. Calcium cyanamide, though not equally effective, has given some satisfactory results in that treated plants showed only a few galls on roots and were decidedly better than controls. But these treatments on a field scale may be out of question for economic reasons. However in limited areas where cost is of no great consideration, the treatment by potassium cyanide *plus* ammonium sulphate may be successful in eradicating soil nematodes under South Indian conditions. Without more extensive trials it would be manifestly unwise to make general recommendations.

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* Seen only in the abstract.

INHERITANCE OF CHARACTERS IN *RAGI, ELEUSINE CORACANA* (GAERTN.) THE FINGER MILLET.

PART VII.—FIST-LIKE EARHEADS.

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(With Plate LXXXI)

In a previous article [Rangaswami Ayyangar *et al*, 1932] the occurrence and inheritance of the three earhead shapes in this millet, *viz.*, 'top-curved', 'in-curved' and 'open', have been dealt with *in extenso*. It was noticed that a factor for density (Q) was present in the curved type and absent in the open type. The 'top-curved' type differed from the 'in-curved' in having a factor E which connoted an elongated rachis. In this scheme of grouping, classification and explanation of genetic behaviour the starting point was the 'in-curved' type, the commonest shape of earhead met with.

At the time the above details were being worked out, there were a few races of *ragis* grown at the Millet Breeding Station which owing to the intensity of the curving appeared roundish, giving the earhead with its compactly 'in-curved' fingers a 'fist-like' appearance. Such heads were called 'fist-like' heads. Some of the vernacular names of this type of earhead are descriptive of this kind of extreme packing. Owing to the paucity of this type of earhead which is not so common in cultivation as the ordinary 'in-curved', it was thought desirable to defer writing about its occurrence and inheritance until more races were forthcoming and wider experience gained.

Fist-like earheads, as has been stated, are characterised by extreme packing. There is practically no central hollow. The number of spikelets per finger is about 73, the average of 15 races through many seasons. This spikelet number is about the general average for *ragi*. What contributes to this extreme packing is a reduction in the length of the rachis, it being 3-5 cm. in length as against the 4-7 cm. of

the 'in-curveds'. The average number of spikelets per centimetre length is 14·8 as against the 11·8 of the 'in-curveds'. In spite of this crowding there is the usual tendency for the tops to be a bit more crowded than the average for the finger.

Segregations between 'in-curved' and 'fist-like' earheads have been met with in the following family (Table I). The table has not been burdened with details of finger length which have been recorded for every one of the plants, similar to those presented in the previous article.

TABLE I.

Family E. C. 925.

Year	Generation	Family No.		Parental character	Progeny behaviour	
					In-curved	Fist-like
1928	F_2		E. C. 925	In-curved	158	48
	F_3	E. C. 925/1	E. C. 1205	In-curved	185	46
		E. C. 925/2	„ 1206	„	172	63
	F_4	E. C. 1206/1	E. C. 1462	In-curved	158	52
		E. C. 1206/2	„ 1463	„	157	70
		E. C. 1206/3	„ 1464	„	181	55
					1,011	334

All the recessive 'fist-like' selections bred pure in all the generations. Extreme care was necessary in the selection of seed material, as the character could be definitely determined only at its optimum manifestation which lasted for a very short period of the life of the crop.

From the above table it will be obvious that the 'fist-like' earhead with its packing and curving has the Q factor in common with the 'in-curveds'. Its individuality has therefore to be sought in some expression of the E factor determining rachis length. That a 'fist-like' earhead is a simple recessive to 'in-curved' shows a monogenic difference between the two. This difference must be independent of the difference between 'in-curved' and 'top-curved'. The necessity, for two E Factors, E_1 and E_2, is thus indicated. The families segregating for 'top-curved', 'in-curved' and 'open', recorded in the previous article, must have been pure for one of the E factors.

In the course of the examination of the 'fist-like' races a natural cross with 'top-curved' fingers was noticed in a 'fist-like' population, and advantage was taken of this to pursue the inter-relationship between the curveds. The history of family E. C. 1337 is presented below.

TABLE II.

Family E. C. 1337.

Year	Generation	Family No.	Parental character	Progeny behaviour			
				Top-curved	In-curved	Fist-like	Open
1929	F_2	E. C. 1337	Top-curved	99	51	13	60
	F_3	E. C. 1337/20 E. C. 1680	Top-curved	53	27	7	27
		,, 1337/27 ,, 1687	,,	71	36	10	37
		,, 1337/29 ,, 1689	,,	71	38	13	37
		,, 1337/10 ,, 1670	,,	71	41	10	...
		,, 1337/4 ,, 1664	,,	89	23	...	23
		,, 1337/5 ,, 1665	,,	103	11	...	36
		,, 1337/6 ,, 1666	,,	65	12	...	19
		,, 1337/7 ,, 1667	,,	50	18	...	22
		,, 1337/15 ,, 1675	,,	84	19	...	26
		,, 1337/17 ,, 1677	,,	60	16	...	25
		,, 1337/22 ,, 1682	,,	76	23	...	34
		,, 1337/28 ,, 1688	,,	103	31	...	43
		,, 1337/11 ,, 1671	,,	43	25	...	..
		,, 1337/19 ,, 1679	,,	65	27	...	...
		,, 1337/21 ,, 1681	,,	69	28	...	...
		,, 1337/26 ,, 1686	,,	90	37	...	...
		,, 1337/9 ,, 1669	,,	85	...	...	31
		,, 1337/13 ,, 1673	,,	75	...	...	28
		,, 1337/16 ,, 1676	,,	65	...	...	25
		,, 1337/30 ,, 1690	,,	133	...	...	38
		,, 1337/1 ,, 1661	,,	pure	...	...	...
		,, 1337/2 ,, 1662	,,	,,	..	..	...
		,, 1337/8 ,, 1668	,,	,,	...	...	...

TABLE I—*contd.*

Year	Generation	Family No.	Parental character	Progeny behaviour			
				Top-cuvred	In-curved	Fist-like	Open
	F_3— *contd.*	E. C. 1337/ 3 E. C. 1663	In-curved	..	77	23	51
		„ 1337/18 „ 1678	„	..	48	8	20
		„ 1337/24 „ 1684	„	..	68	11	36
		„ 1337/25 „ 1685	„	..	44	13	20
		„ 1337/42 „ 1692	„	..	79	21	36
		„ 1337/44 „ 1694	„	..	74	19	33
		„ 1337/51 „ 1701	„	..	51	12	35
		„ 1337/14 „ 1674	„	..	76	26	..
		„ 1337/45 „ 1695	„	..	103	32	..
		„ 1337/46 „ 1696	„	..	78	27	..
		„ 1337/47 „ 1697	„	..	61	18	..
		„ 1337/65 „ 1703	„	..	53	12	..
		„ 1337/12 „ 1672	„	..	46	..	20
		„ 1337/41 „ 1691	„	..	77	..	34
		„ 1337/23 „ 1683	„	..	pure	..	..
		„ 1337/43 „ 1693	„	..	„	..	..
		„ 1337/48 „ 1698	„	..	„	..	..
		„ 1337/49 „ 1699	„	..	„	..	..
		„ 1337/50 „ 1700	„	..	„	..	..
		„ 1337/52 „ 1702	„	..	„	..	..
		„ 1337/67 „ 1705	Fist-like	..	..	69	32
		„ 1337/66 „ 1704	„	..	..	pure	..
		„ 1337/68 „ 1706	„	..	..	„	..

An examination of the 46 families of the third generation in the above table reveals beyond doubt the existence of two factors determining rachis length, *viz.*, E_1 and E_2. Either of these two gives an 'in-curved'. When neither is present the earhead is 'fist-like'. When both are present the earhead is 'top-curved'. It is

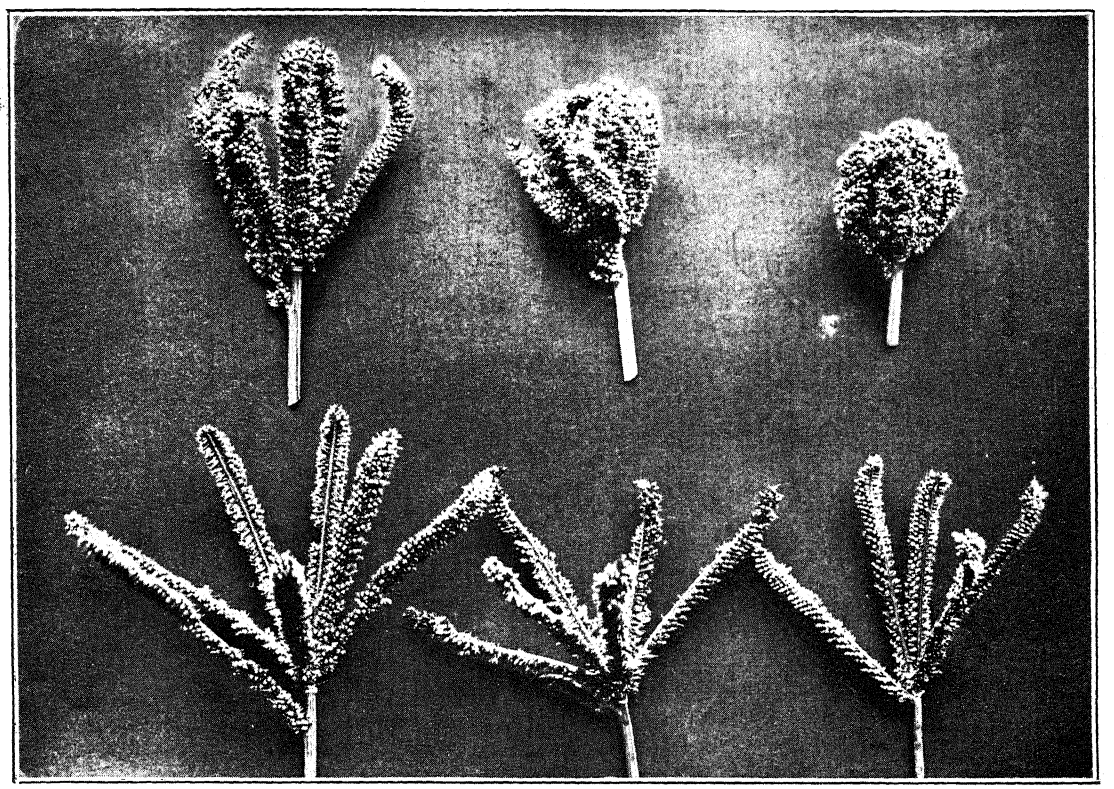
needless to add that all the above have the Q factor, and that in its absence the earheads are 'open' and range themselves into the corresponding 'long opens', 'short opens', and 'very short opens' (Plate LXXXI). On this factorial basis the third generation should consist of the following, their genetic constitutions and expected ratios being as follows :—

TABLE III.

Interplay of factors Q, E_1 and E_2.

Generation	Genetic constitution	Phenotypic expression	Progeny ratio			
			Top-curved	In-curved	Fist-like	Open
F_2	$QqE_1e_1E_2e_2$ . . .	Top-curved	27	18	3	16
F_3	$QqE_1e_1E_2e_2$ . . .	Top-curved	27	18	3	16
	$QqE_1e_1E_2E_2$ . . .	,,	9	3	..	4
	$QqE_1E_1E_2e_2$ . . .					
	$QQE_1e_1E_2e_2$ . . .	,,	9	6	1	..
	$QqE_1E_1E_2E_2$ . .	,,	3	..	..	1
	$QQE_1e_1E_2E_2$. . .	,,	3	1	..	..
	$QQE_1E_1E_2e_2$ . . .					
	$QQE_1E_1E_2E_2$ . .	,,	pure	..	..	..
	$QqE_1e_1e_2e_2$ . . .	In-curved	..	9	3	4
	$Qqe_1e_1E_2e_2$ . . .					
	$QqE_1E_1e_2e_2$ . . .	,,	..	3	..	1
	$Qqe_1e_1E_2E_2$ . . .					
	$QQE_1e_1e_2e_2$ . . .	,,	..	3	1	..
	$QQe_1e_1E_2e_2$ . . .					
	$QQE_1E_1e_2e_2$ . . .	,,	..	pure	..	..
	$QQe_1e_1E_2E_2$ . . .					
	$Qqe_1e_1e_2e_2$ . . .	Fist-like	..	..	3	1
	$QQe_1e_1e_2e_2$ . . .	,,	..	..	pure	..

Top-curved Incurved Fist-like



Long open Short open Very short open

ELEUSINE CORACANA (GAERTN.)—EARHEAD SHAPES.

TABLE III—*contd.*

Generation	Genetic constitution	Phenotypic expression	Progeny ratio			
			Top-curved	In-curved	Fist-like	Open
	$qqE_1e_1E_2e_2$ . . .	Open	..	..	..	All
	$qqE_1e_1E_2E_2$ . . .					
	$qqE_1E_1E_2e_2$ . . .					
	$qqE_1E_1E_2E_2$ . . .					
	$qqE_1e_1e_2e_2$ . . .					
	$qqE_1E_1e_2e_2$ . . .					
	$qqe_1e_1E_2e_2$ . . .					
	$qqe_1e_1E_2E_2$ . . .					
	$qqe_1e_1e_2e_2$ . . .					

It is remarkable that all the above segregations have been met with in the expected manner in family E. C. 1337.

To put these phenomena to a final test, an artificial cross between a 'very short open' (the new type of 'open', allelomorphic to 'fist-like' earheads) and a 'top-curved' was made in Cross No. CCXXVIII and the first generation was a 'top-curved', which in the second generation segregated into 200 'top-curved', 104 'in-curved', 22 'fist-like', and 97 'opens' of varying length, a good 27 : 18 : 3 : 16 ratio.

Extracted types in all the six groups (3 'curveds' and 3 'opens' from E. C. 1337) have been carried forward and the measurements of finger length in a population of 200 in each is given below.

TABLE IV.

Finger length measurements of genetic groups of earheads.

E. C. No.	Panicle shape	Finger length in cm.													
		3	4	5	6	7	8	9	10	11	12	13	14	15	16
1662	Top-curved .	..	..	..	3	44	143	10	..	..	..	..	..	..	..
2112	In-curved .	..	15	153	31	1	..	..	..	..	..	..	..	..	..
1704	Fist-like . .	60	129	11	..	..	..	..	..	..	..	..	..	..	..
2111	Open (long) .	..	..	..	..	..	..	..	3	11	54	89	29	12	2
2110	Open (short) .	..	..	..	..	..	5	31	76	72	15	1	..	..	..
2113	Open (very short)	..	..	..	..	28	113	53	6	..	..	..	..	..	..

The independence of the factors determining panicle shape from those responsible for plant purple pigmentation has been recorded in Table VI of the previous paper on earhead shapes.

Even with the elaboration of the E factor into E_1 and E_2, the above independence has been maintained, P and E factors (1 or 2) giving a simple dihybrid ratio (E. C. 925).

Family E. C. 925.

Purple plant		Green plant	
In-curved	Fist-like	In-curved	Fist-like
124	38	34	10

The relation of Q and E factors determining panicle shape have been worked in conjunction with C_x factor determining the depth of green in the unripe pericarp. The following family proves that they are independent.

Family E. C. 2414.

	Top-curved		In-curved		Fist-like		Open	
	Green	Light green	Green	Light green	Green	Light green	Green	Light green
	152	48	82	22	17	5	75	22
Expected ratio	81	27	54	18	9	3	48	16

They have also been worked with the B factors for grain colours and found to be similarly independent.

In the case of the brown grain of this millet its factorial composition could not be resolved in its entirety, till the comparatively minor race of white grains crossed with the more common brown grains [Rangaswami Ayyangar, *et al*, 1931]. Similarly through crosses with the comparatively rare 'fist-like' races, the E factor has been enabled to be resolved into E_1 and E_2. The 'fist-like' earhead while it has a compact and economic build has possibly its geographical and climatic limitations different from the environmental factors determining the distribution of open-headed

varieties, and it is therefore no wonder that the 'top-curved' and 'in-curved', which represent a happy mean, are widely represented in the common varieties of this millet.

Summary.

The earheads of *ragi* vary in their length. Two factors E_1 and E_2 determine this elongation. Either of them gives a 'short' length, both give a 'long' length. When neither is present a 'very short' length is obtained.

A factor Q determining the density of disposition of spikelet per centimetre length results in a crowding and consequent curving of the earheads, leading to the three types of curved earheads, *viz.*, 'top-curved', 'in-curved', and 'fist-like'. The number of spikelets in the earheads of *ragi* being about equal, such crowding naturally reacts on length. In the absence of Q the corresponding 'opens' are 'long open', 'short open' and 'very short open'.

Factors Q, E_1 and E_2 are independent of P, I_1 and I_2 (plant purple pigmentation), B_1, B_2 and S (grain colour), and C_x (unripe pericarp colour) factors.

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INHERITANCE OF CHARACTERS IN *RAGI, ELEUSINE CORACANA* (GAERTN.).—THE FINGER MILLET.

PART VIII.—EARHEAD COLOUR FACTORS.

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In a previous article [Rangaswami Ayyangar and Krishna Rao, 1931] three major types in purple pigmented plants have been described and their inheritance worked out. It was then indicated that there were other variations under further study. It is needless to stress the fact that the identification of the various types is a necessary preliminary in all selection, classification and hybridization schemes. This article describes a fourth type, designated "medium purple".

In this type of plant the earhead is coloured light similar to the head in the dilute purple. It differs from the dilute purple in having its body pigmentation as good as in the common purple. The status of this in the pigmented group is midway between purple and dilute purple.

That there could be a separation in the manifestation of purple pigmentation on the earhead on one side and in the plant body on the other was evident from the localized purple already dealt with. It was then thought that it could be an extreme case of dilution, a degree one gene removed from the dilute purple.

The occurrence of this new type, medium purple, clearly indicates that there is a definite difference between the factors responsible for purple pigmentation in general and its dilution, as opposed to its definite localization.

Definite factors seem to be responsible for the depth of manifestation of purple pigmentation on the earhead. These have been found to be two in number and are designated H_1 and H_2. In the types of earheads whose inheritance has already been recorded, all of them seem to have been pure for one or both of these H factors.

The gathering together of various types of *ragi* brings into the collection different genetic types, one of which, *viz.*, E. C. 141, is primarily responsible for bringing out the existence of a second H factor. This E. C. 141 is one of the minority groups of 'fist-like' earheads and its dwarf nature and build afforded little opportunities for noticing the fact that, whereas the pigmentation of its vegetative parts was of the normal type of purple, that on the earhead was of the dilute type. This fact was not noticed until a natural cross occurred in it, which, when carried forward, segregated into earheads of two depths of purple. The new type of pigmentation with good vegetative purple and dilute earhead purple was thus noticed and in due course the extracted recessive was fixed in E. C. 1810 and kept going as a new type 'medium purple'.

To determine the affinities between this new type, 'medium purple', and the other three types, purple, dilute purple and localized purple, crosses were made and carried through three generations. All the segregations met with between this medium purple and the other three types are summarised below.

TABLE I.

Purple and medium purple (3 : 1).

Family No.	Purple	Medium purple
E. C. 1804	84	28
E. C. 1805	86	30
E. C. 1808	129	41
E. C. 2352	194	53
E. C. 2358	171	59
E. C. 2360	181	66
E. C. 2368	145	49
E. C. 2369	156	55
E. C. 2371	175	48
E. C. 2372	122	37
E. C. 2380	168	50
E. C. 2382	155	66
E. C. 2383	166	52
E. C. 2386	200	81
	2,132	715

The difference between 'purple' and 'medium purple' as evidenced from the above is monogenic, 'medium purple' being recessive.

In the following segregations, the digenic nature of the difference in the depth of pigmentation in the earhead in evident :—

TABLE II.

Purple and medium purple (15 : 1).

Family No.	Purple	Medium purple
E. C. 2237	223	16
E. C. 2238	261	14
E. C. 2367	145	9
E. C. 2370	198	13
E. C. 2375	159	11
E. C. 2379	224	15
E. C. 2384	163	13
	1,373	91

It will be obvious that two factors H_1 and H_2 are, as stated above, responsible either alone or together for the depth of manifestation of purple pigmentation in the glumes of the *ragi* earhead. The slight increase in depth in their concurrent presence is so imperceptible that it is not easily separable from a single H manifestation.

'Medium purple' behaves as a simple dominant to 'dilute purple' as will be seen from the following table :—

TABLE III.

Medium purple and dilute purple.

Family No.	Medium purple	Dilute purple
E. C. 2402	124	41
E. C. 2403	113	42
E. C. 2405	153	38
	390	121

This is simply the dilution factor at work, diluting the body pigment. These dilute purples have no H factor.

'Medium purple' behaves as a simple dominant to 'localized purple' as the following families show :—

TABLE IV.

Medium purple and localized purple.

Family No.	Medium purple	Localized purple
E. C. 2363	137	30
E. C. 2365	199	55
E. C. 2366	159	55
	495	140

The 'localized purples' of this table lack H factors.

Crosses have been designed between suitable parents bringing together 'medium purple' on the one hand and the dilute or localized purple with the H factor (recessives to purples) on the other with the resultant F_1 plants having ordinary purple glumes and the F_2 giving the expected 9 : 3 : 4 ratios (Tables V and VI).

TABLE V.

Medium purple × *dilute purple* (*with H*).

F_1	Purple		
F_2	Purple	Medium purple	Dilute purple
E. C. 2400	150	45	56

TABLE VI.

Medium purple × *localized purple* (*with H*).

F_1	Purple		
F_2	Purple	Medium purple	Localized purple
E. C. 2236	156	36	55
E. C. 2353	177	65	81
E. C. 2357	170	63	79
	503	164	213

The above tables go to show that the H factor should have come in through the dilute purple and localized purple parents, only, in these cases the H factor lacked expression in the general dilution and localization, whittling down a good show of colour in general, in these light-coloured earheads.

An attempt was made to single out a type, *viz.*, E. C. 106, a localized purple whose glumes were a shade deeper than the rest of localized purples and cross it on to a medium purple. The F_1 proved a purple and the F_2 segregated into the broad groups purple and localized purple, the purples being separable with difficulty into purple and medium purples indicative of a rough 15 : 1 ratio. This experience shows that one of the possible effects of an H_1, H_2 presence in dilute purples and localized purples is a slight deepening of the tint of the pulled-down purple, characteristic of the earhead in these two groups.

This point is mentioned as showing the possibilities of a sub-grouping under the major genetic groups, but the difficulty of constant and clear-cut phenotypic expression makes the pursuit of this clue a difficult proposition.

SUMMARY.

Two factors H_1 and H_2 determine depth of manifestation of purple pigmentation in the glumes of the *ragi* earhead. These may be operative either alone or together.

Their effect in the absence of one or both the I factors is negligible.

REFERENCE.

Rangaswami Ayyangar, G. N. and Krishna Rao, P. (1931). *Ind. J. Agric. Sci.* **1**, 434.

A SIMPLE METHOD FOR THE FORECAST OF EMERGENCE OF LAC LARVÆ, AND A DESCRIPTION OF THE MYOLOGY OF THE ADULT FEMALE LAC INSECT, *LACCIFER LACCA* KERR. (COCCIDÆ).

BY

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(With Plates LXXXII and LXXXIII)

In lac cultivation the judgment of the state of maturity of the lac crop and of the date of emergence of lac larvæ is a matter of great importance. The early removal of the crop from the trees does not allow the females to get full nourishment and therefore, without food, the vitality of the females goes down considerably, with the result that each individual female is rendered almost unfit for the delivery of hundreds of eggs. Cutting off the food supply of the females by the early removal of the crop from the trees also indirectly weakens the developing embryos and, therefore, the few eggs that are laid by such females die a premature death. On the other hand if the crop is removed from the trees when the emergence of larvæ actually starts in the crop, there may not be time to harvest the crop and infect the new trees with the selected brood lac, before a large number of larvæ have swarmed out and died for want of food, this would naturally lead to a poor future crop.

The ryot cultivator, specially in Bihar and Orissa, is to some extent able to forecast the date on which the lac larvæ will swarm fairly accurately, by the yellow appearance of the lac incrustation, but the ryot is unaware of the real nature of this appearance of the lac incrustation and often has to rely on long experience in lac cultivation to make a fairly accurate guess. To solve this important difficulty, Misra [1928] evolved a method of forecasting the emergence of lac larvæ. This method is based on the ovarian development. The method though scientific and most up-to-date has three drawbacks :—

(1) It requires the use of the microscope which in Misra's own words is "beyond the reach of an ordinary grower".

(2) It gives accurate forecasts for the emergence of lac larvæ from the *Katki* (July-November), *Baisakhi* (November-July), and *Jethwi* (February-

July) crops but not from the *Aghani* (July-February) crops. Because due to low and fluctuating temperatures the ovarian development in the *Aghani* crops is not steady and does not accurately correlate with periods given for the emergence of lac larvæ—in explanation of his Plate XI.

(3) The figure 9 of Plate XI of his bulletin which shows the shape of embryos 8 to 12 days before the emergence of larvæ is readily recognisable, but the next figure (10) which represents the shape of the "embryos" in the ovary 5 days before the emergence of larvæ is not easy to recognise. Moreover, in the majority of females of every crop eggs are delivered in the incubating chamber at this time and as Misra has not recorded this fact a lay examiner of the ovary in the absence of this statement might be confused by the presence of eggs and larvæ in the incubating chamber outside the body of the insect and the presence of "embryos" in the ovary.

It is these last 8 to 12 days which are important as harvesting the crop within this period would be beneficial, and, therefore, the most useful system of forecast of emergence would be the one which is not expensive and gives reliable and easily recognisable stages within this period.

In view of the above necessity investigation of the lac incrustation and the working of the perivaginal glands was taken up in 1927 and a method of forecast has been evolved which overcomes the difficulties of Misra's method and can be followed by a cultivator even with the naked eye, though a magnifying glass magnifying 4 to 6 times is a useful aid.

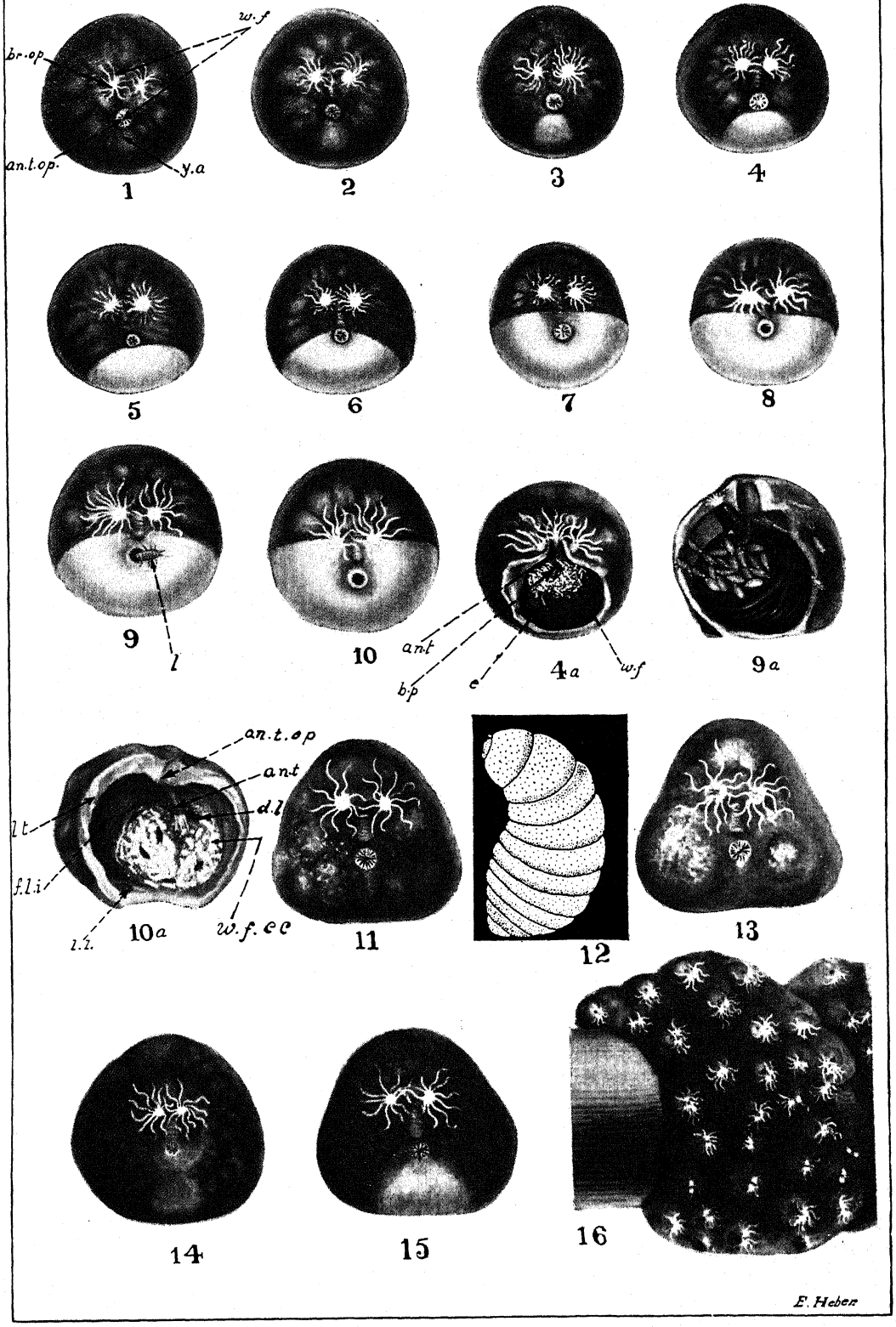
This method of forecasting the emergence of lac larvæ is based on the appearance of a yellow spot (Plate LXXXII) due to muscular contraction of the female lac insect as summarised by Negi [1929] under mechanism of emergence of larvæ, and under perivaginal glands and forecast of emergence in the Annual Reports (1928-29, 1929-30 and 1930-31) of the Indian Lac Research Institute. A preliminary summary of these observations and results is published by Glover [1931].

To understand the details and mechanism of this method of forecast of emergence, it is necessary to describe first the myology of the female lac insect and the general appearance and position of the perivaginal glands.

Myology and perivaginal glands of the adult female lac insect.

Myology.

The musculature of the adult female lac insect due to its fixed habit of life in a resinous test is greatly modified. Misra [1928] has figured the musculature but



(For explanation see p. 1094.)

has not described it. Negi [1929] described and figured only the tergo-sternal and posterior sternal muscles which are most relevant to the deposition of eggs and emergence of larvæ. Misra [1930, 1931] has to some extent described and figured the musculature. But these descriptions and figures do not meet the purpose of this paper and, therefore, a brief description of the muscular system and illustration of the changes it undergoes during the period of egg-laying and the emergence of larvæ from the lac test is given.

Negi in a paper " The Alimentary Canal, its Appendages, Salivary Glands and the Nervous System of the Adult Female Lac Insect " under publication has stated that the lac females assume two shapes, one circular and the other pyriform. Whatever be the shape of the female, the number of the tergo-sternal, sternal and tergal muscles remains the same and only the angle of attachment of the tergo-sternal muscles from the dorsal to the ventral body wall changes (Plate LXXXIII, figs. 17*a*, 26).

Tergo-sternal muscles.—There arise six bands of anterior tergo-sternal muscles (Plate LXXXIII, figs. 17*a*, 25 ; a. t. s. m.) from below the posterior end of the brachia of either side and in circular females they are symmetrically attached a little behind the mouth parts close to the posterior end of the first sternal, the second sternal and the anterior end of the third sternal muscles (Plate LXXXIII, fig. 17*a* ; a. t. s. m.). But in the pyriform females, the insertion of the anterior tergo-sternal muscles to the ventral body wall being at an acute angle, the first sternal muscle is practically not covered by them (Plate LXXXIII, fig. 26). From the posterior end of the pedicel of the dorsal spine (Plate LXXXIII, fig. 25 ; d. s.) on either side arise three bands of posterior tergo-sternal muscles. The anterior two (p. t. s. m. 1, 2) are symmetrically attached immediately outer to the junctions of the fifth and sixth sternal muscles. The third band of the posterior tergo-sternal muscles (p. t. s. m. 3) of either side is attached a little in front of the anal tubercle at a distance from the sixth sternal muscles. The fourth tergo-sternal muscles (p. t. s. m. 4) of either side consists of two or more than two bands and originate from in front of the anal tubercle and partly lie on the ventral body wall outside and parallel to the seventh sternal muscles which are situated on either side of the vaginal opening (birth pore b. p.).

Sternal muscles.—There are eight sets of sternal muscles. They lie on the ventral body wall and extend from a little behind the mouth parts and end in the anal tubercle (Plate LXXXIII, fig. 25 ; s. m. 1-8). The first set of the sternal muscles generally consists of four bands and the remaining of six to eight bands. The fifth sternal muscles are the longest.

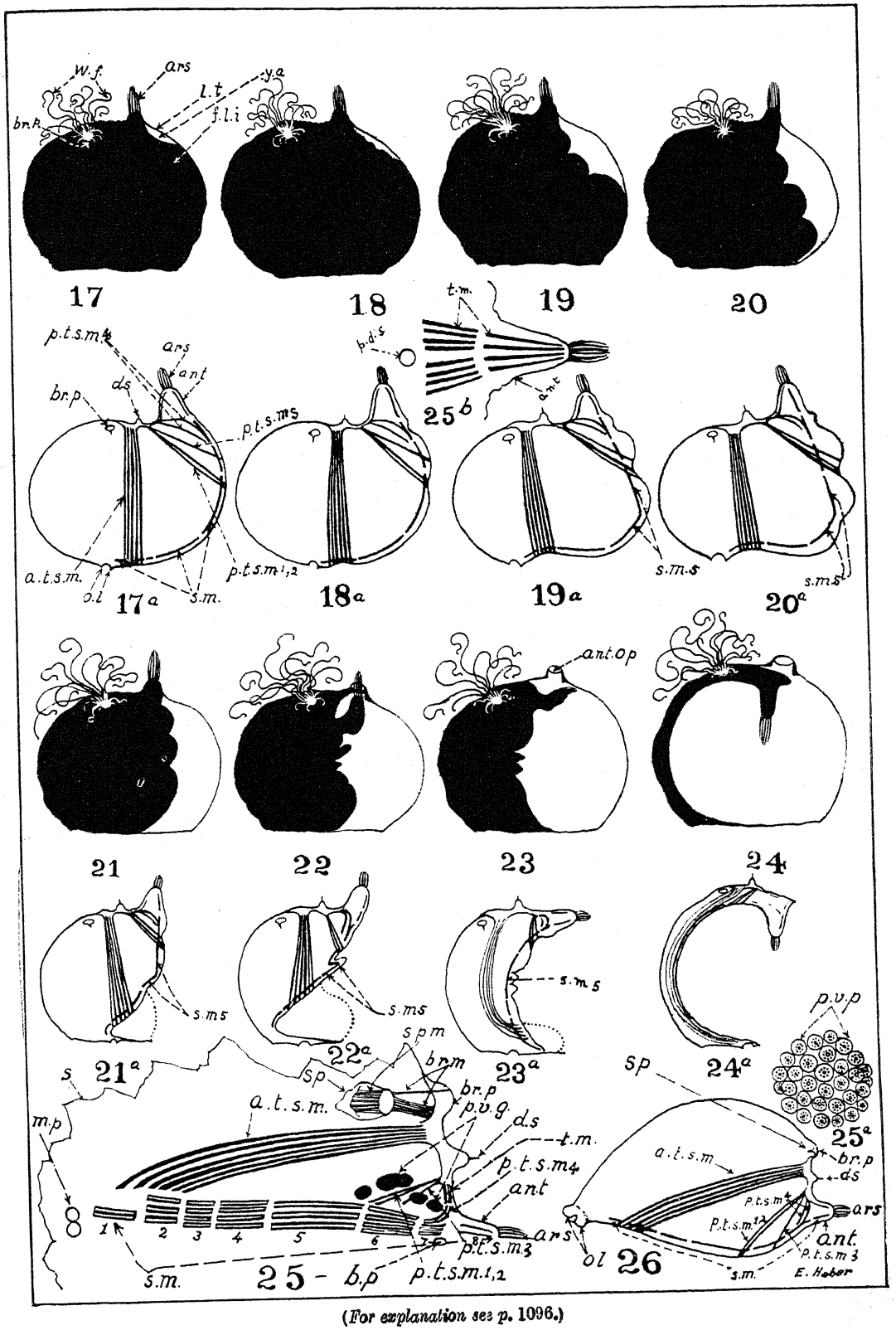
Tergal muscles.—In the adult female only a few tergal muscles are present. Four bands of tergal muscles run into the anal tubercle, and four bands (Plate

LXXXIII, figs. 25, 25*b*, t. m.) extend on either side from the base of the anal tubercle to a level with the origin of posterior tergo-sternal muscles. A band of brachial muscles from between the anterior end of the brachial plate and a number of them (brm) from the posterior end of the brachium of each side run to the spiracle. A number of closely set spiracular muscle bands (spm) run from the posterior to the anterior end of the spiracle.

Perivaginal glands.—On either side in front of the anal tubercle an oblique row of the perivaginal glands (Plate LXXXIII, fig. 25, p. v. g.) is situated on the ventral body wall. In each row, there are 8-12 clusters of glands and each cluster opens out by pores (Plate LXXXIII, fig. 25*a* ; p. v. p.) which present a honeycombed appearance. The histology and other details of these glands are out of the scope of the paper. But, it is necessary to repeat the statement made by Negi as regards *L. lacca* [1929] that these glands deposit wax filaments into the incubating chamber beneath them and [1928-29] that the beginning of the egg-laying period in a female lac insect has a close relationship to the wax producing activity of these glands.

Yellow area and its relation to the muscular contraction of the female lac insect and its perivaginal glands.

Usually at the base of anal tubercular on the posterior side of a nearly fully developed individual lac test is found a small deep yellow spot (Plate LXXXII, fig. 1, and Plate LXXXIII, fig. 17 ; y. a.). This is so, because the body of the lac insect which is dark crimson in colour is slightly apart from the translucent orange lac test of a full grown female at this place. When the female is full grown and the time for oviposition nears, the anterior and posterior tergo-sternal muscles (Plate LXXXXIII, fig. 17*a* ; a. t. s. m., p. t. s. m. 1-4) contract a little and the ventral body wall at the place of their attachment is drawn towards the dorsal (Plate LXXXIII, fig. 18*a*), and the female in the resinous test if exposed at the posterior end would look as in Plate LXXXIII, fig. 18, and the deep yellow area increases as shown in Plate LXXXII, fig. 2. The yellow area caused ventrally by the contraction of the anterior tergo-sternal muscles is not visible either in the posterior or side view. The next contraction in the tergo-sternal muscles (Plate LXXXIII, fig. 19*a*) leads to a bend in the fifth sternal muscle (s. m. 5) and to a further increase in the deep yellow area (Plate LXXXII, fig. 3). The posterior part of the female assumes the shape shown in Plate LXXXIII, fig. 19. The third contraction of the tergo-sternal muscles leads to a further bend in the fifth sternal muscle (Plate LXXXIII, fig. 20*a*) and an increase in the yellow area (Plate XXXII, fig. 4) and the female inside the cell assumes the form shown in Plate LXXXIII, fig. 20. It will be seen that the deep yellow area has now



(For explanation see p. 1096.)

lightened to yellow in this stage, due to the fact that the perivaginal glands have begun to secrete the white wax filaments and the female starts to lay eggs (Plate LXXXII, fig. 4*a*; e., w. f.). In the fourth contraction the tergo-sternal muscles (Plate LXXXIII, fig. 21*a*) make the female look as in Plate LXXXIII, fig. 21 and, due to a further deposition of eggs, secretion of more wax filaments by the perivaginal glands and the accumulation of the empty egg cases and the larvae in the yellow area makes it look bright yellow and increased in size as shown in Plate LXXXII, fig. 5. The tergo-sternal muscles (Plate LXXXIII, fig. 22*a*) continue contracting with the result that the fifth sternal muscle (s. m. 5) becomes doubly bent upon itself and becomes S-shaped, this leads to a partial withdrawal of the anal tubercle from the anal tubercular opening of the test and formation of a passage between the anal tubercular opening and the incubating chamber. The female in the test assumes the shape shown in Plate LXXXIII, fig. 22, and the bright yellow area as in Plate LXXXII, figs. 6-7. The succeeding contractions in the tergo-sternal muscles alter the shape of the female and the angle of their attachment to the ventral and dorsal body wall so much that the fifth sternal muscle is straightened (Plate LXXXIII, fig. 23*a*; s. m. 5) and the anal tubercle is completely withdrawn from the anal tubercular opening (anal orifice) in the lac test; and the female in the test looks as in Plate LXXXIII, fig. 23, and the bright yellow area as in Plate LXXXII, fig. 8. The contractions in the tergo-sternal muscles (Plate LXXXIII, fig. 24*a*) and the subsequent increase in the bright yellow area (Plate LXXXII, figs. 9-10) continue till the female is reduced to a what might be described as a lining to the anterior half of its test (Plate LXXXIII, fig 24) and the bright yellow area has assumed the shape shown in Plate LXXXII, fig. 10; and if a cell is opened at this stage it will be found to contain the powdered wax filaments, empty egg cases and some dead and a few living larvæ (Plate LXXXII, fig. 10*a*).

FORECAST OF EMERGENCE OF LAC LARVÆ.

Having described above the musculature and perivaginal glands of the female insect and their import on the method of forecast of emergence, a brief description of the manner in which the method is to be followed is given below.

General signs of crop maturity may be judged by the following three tests :—

1. Granular appearance of the embryos :—If a female cell is crushed between the fingers, the developing embryos in the ovary look granular about 3-4 weeks before the emergence of larvæ in the *Katki* (July-November), *Baisakhi* (November-July) and *Jethwi* (February-July) and *Aghani* (July-February) crop. The embryos become more and more prominent as the time for swarming nears.

2. *Appearance of cracks in the incrustation*:—The lac incrustation begins to crack about 2-3 weeks before swarming in the *Katki*, *Baisakhi* and *Jethwi* crops and about 4-5 weeks in the *Aghani*.

3. *Appearance of the incrustation and its peeling from the host.*—In all the crops the incrustation presents a drier appearance about two weeks before the emergence of larvæ is due and it can be peeled off from the host twig with a greater ease.

Accurate forecast of emergence of larvæ by examination of the orange yellow area in the female test.

However the growth of the yellow orange spot in the female test is the only index which enables one to forecast accurately the dates of emergence of larvæ in all the crops, provided the day temperature does not fall below 20°C. (68°F.). Because the female ceases to lay eggs if the temperature either at the advent of or during the oviposition period falls below 17°C. (62·6°F.), and the larvæ do not come out of the lac test if the temperature is below 20°C. (68°F.) [Negi, Misra and Gupta, 1931; and Glover, Negi, Misra and Gupta, 1932]. The low temperature contingency, however, presents itself only during the *Aghani* (July-February) crops.

On the dorsal surface of every lac test there are three holes from which white wax filaments project. These holes are situated in a manner that they form from an isosceles to an equilateral triangle (Plate LXXXII, fig. 1). Two out of these three holes called the brachial openings (br. op) are subequal in size and do not look like holes with the naked eye but wax filaments are seen protruding from them. The third hole the anal tubercular opening (Plate LXXXII, fig. 1; an. t. op.) is much bigger in size and prominent. A thick bunch of white filaments surrounding the anal setae projects from it. It is this opening which gives a clue to the location of the yellow spot and serves as a guide to the method of forecast of emergence. In order to ascertain that one is looking at the anal tubercular opening of an insect sufficiently healthy to lay eggs, the tube of white filaments (Plate LXXXIII, figure 17; ars) should be touched with a needle. If the insect is healthy the touch will bring out at the top of the tube a drop of liquid excreta called the 'honeydew'. If the drop of the 'honeydew' is not forthcoming from the anal tubercle even after repeated touches then the anal tubercular opening of another test should be sought and the test repeated in order to be sure that one is looking at the anal tubercular opening of a cell which is capable of laying eggs. Now, if the anal tubercular opening is taken as the apex of the triangle referred to above, the brachial openings will be found to form the base of the triangle.

The brachial openings are situated towards the anterior end, and the growth of the yellow area takes place only up to them.

The relative position of the three apertures and the yellow area on the test of an insect even in thick incrustation is more easily spotted if one gets experience by beginning observations on the isolated growing or less coalesced cells. And in every crop isolated or less coalesced cells should be preferred to forecast the emergence. They are found in abundance in the *Katki*, *Baisakhi*, and also in the *Jethwi* and *Aghani* crops grown on host plants other than *kusum* (*S. trijuga*). Even in *kusum*, such cells can be picked out after a little search. The cell should always be examined from the posterior side.

Having decided upon suitable cells the posterior end of the tests (the part of the test lower to the anal tubercular opening) should be freed from the coating of dust or fungus by a gentle rub of a finger and a deep yellow spot (y.a.) immediately below the anal tubercular opening (Plate LXXXII, fig. 1) looked for from the posterior side and compared to figs. 2-10 in Plate LXXXII. The yellow area gradually increases first on the side posterior to anal tubercular opening and then on either side anterior to it till it becomes in level with the brachial openings and generally stops growing further. The reasons for the growth of the yellow area and changes in its colour have been explained in the previous pages.

In the end it may be said that certain cells, which are either parasitised or dead or unhealthy, show the yellow spots resembling those illustrated for the forecast of emergence. It would, therefore, be better that the cells after examination are crushed and examined to discover whether they are healthy. Also that the *S. trijuga* lac cells (Plate LXXXII, fig. 14) due to the compact growth of resin are slightly different in shape than the cells growing on other host plants, therefore, the deep yellow spot in the *S. trijuga* lac cells is seen a little lower down on the test than in the cells of other broods. In some localities in addition to the crimson lac insect yellow insects are found, and, due to the very similar colour of the yellow insect and its orange test, the yellow area on the test is not noticed easily and therefore a more careful observation is necessary.

To forecast the emergence of larvæ in a crop (Plate LXXXII) and its explanation should be used in conjunction with Table II. All the lac cells even on the same twig are not found in the same stage of development, therefore, the lac crops as stated in the foot notes of Table II and especially the portion of the crops to be used as brood lac for the next infection should not be removed from the trees before a few cells at least have reached stage 4 or 5 on Plate LXXXII; by doing so, most of the cells in the crops will have reached the stages when the vitality of the majority of the insects and their young will not be affected.

This method of forecast of emergence was put to repeated tests and the results of such tests compiled in Table I will show that it gives fairly accurate forecasts.

I wish to express my thanks to Mr. P. M. Glover, the Entomologist at the Institute, for criticisms on the draft of the paper and to Mr. E. Heber, the Artist, for drawing the illustrations accompanying it.

TABLE I.

Determination of larval emergence on development of yellow spot.

Crop	Specimen No.	Date of examination	Reference figure	Date of emergence according to examination of yellow area	Actual date of emergence
Katki (July-Nov.)	1	3rd Oct.	2	9th-15th Oct.	13th Oct.
	2	6th Oct.	2	12th-18th Oct.	17th Oct.
	3	9th Oct.	3	13th-16th Oct.	13th Oct.
	4	10th Oct.	3	14th-17th Oct.	17th Oct.
	5	26th Oct.	4	30th Oct.-1st Nov.	30th Oct.
	6	9th Oct.	5	12th-13th Oct.	13th Oct.
	7	12th Oct.	5	15th-16th Oct.	15th Oct.
	8	13th Oct.	6	15th-16th Oct.	15th Oct.
	9	22nd Oct.	8	23rd Oct.	23rd Oct.
Aghani (July-Feb.)	10	19th Jan.	2	31st Jan.-8th Feb.	31st Jan.
	11	12th Jan.	2	24th Jan.-1st Feb.	30th Jan.
	12	27th Jan.	2	8th-15th Feb.	8th Feb.
	13	9th Jan.	3	17th-27th Jan.	25th Jan.
	14	27th Jan.	4	3rd-10th Feb.	4th Feb.
	15	27th Jan.	4	3rd-10th Feb.	3rd Feb.
	16	20th Jan.	5	23rd-27th Jan.	24th Jan.
	17	20th Jan.	6	22nd-25th Jan.	23rd Jan.
	18	30th Jan.	7	31st Jan.-1st Feb.	31st Jan.
Baisakhi (Oct.-July)	19	24th June	2	29th June-4th July	4th July
	20	25th June	2	30th June-5th July	2nd July
	21	27th June	4	30th June-3rd July	30th June
	22	30th June	5	2nd-3rd July	3rd July
	23	29th June	7	30th June-1st July	30th June
	24	2nd July	8	3rd July	3rd July
	25	3rd July	8	4th July	4th July
Jethwi (Feb.-July)	26	24th June	2	29th June-6th July	29th June
	27	24th June	2	29th June-6th July	3rd July
	28	25th June	3	29th June-6th July	29th June
	29	29th June	5	1st-2nd July	1st July
	30	24th June	6	25th-27th June	26th June

TABLE II.

Determination of emergence of larvæ from examination of the growth of the yellow spot.

(Plate LXXXII, figs. 2-8.)

Crop	Period for emergence to start from each stage	Fig. 2	Fig. 3	Fig. 4*	Fig. 5†	Fig. 6	Fig. 7	Fig. 8
Katki (July-Nov.)	Average period in round numbers.	8 days	7 days	5 days	4 days	3 days	1 day	Within 24 hrs.
	Common variation period.	6-12 days	4-7 days	4-6 days	3-4 days	2-3 days	1-2 days	Within 24 hrs.
Aghani (July-Feb.)	Average period in round numbers.	16 days	13 days	9 days	5 days	4 days	2 days	Within 24 hrs.
	Common variation period.	12-20 days	8-18 days	7-14 days	3-7 days	2-5 days	1-2 days	Within 24 hrs.
Baisakhi (Nov.-July) and *Jethwi* (Feb.-July)	Average period in round numbers.	7 days	5 days	4 days	2 days	2 days	1 day	Within 24 hrs.
	Common variation period.	5-10 days	4-7 days	3-6 days	2-3 days	1-3 days	1-2 days	Within 24 hrs.

* The secretion of wax filaments by the perivaginal grands and the deposition of eggs by the mother begins in this stage. And in fairly large areas the *Katki*, *Baisakhi* and *Jethwi* crops should be removed at this time.

† The *Aghani* crop should be removed at this time if the weather is favourable, otherwise in the next stage.

SUMMARY.

1. The paper describes the tergo-sternal, sternal and tergal muscles of the full grown female lac insect.

2. The relation of the tergo-sternal and sternal muscles and wax producing activity of the perivaginal glands to oviposition in the female is dicussed.

3. The muscular contraction of the female insect at oviposition is shown to be regular and an easy system of forecast of emergence of lac larvae is described and illustrated.

The system of forecast of emergence is based on the growth of the yellow area and changes in its colour due to the withdrawal of the insect body from the orange coloured test, deposition of white wax filaments by the perivaginal glands and of eggs by the female in the test.

Key to lettering in Plates.

an. t	Anal tubercle
an. t. op	Anal tubercular opening
ars	Anal ring setae
a. t. s. m.	Anterior tergo-sternal muscles
b. p.	Vaginal opening (birth pore)
brm.	Brachial muscles
brp	Brachial plate
br. op	Brachial opening
d. l.	Dead larva.
d. s.	Dorsal spine
e.	Egg
fl. i.	Female lac insect
l.	Larva
l. l.	Living larva
l. t.	Lac test
m. p.	Mouth parts
o. l.	Oral lobes
p. d. s.	Pedicel of dorsal spine
p. t. s. m. 1-3	Posterior tergo-sternal muscle, one to three bands
p. v. g.	Perivaginal glands
p. v. p.	Perivaginal pore cluster
s.	Skin
s. m. 1-8	Sternal muscles, one to eight
sp.	Spiracle
sp. m.	Spiracular muscles
t. m.	Tergal muscles
w. f.	Wax filaments
w. f. e. c.	Wax filaments and empty egg cases
y. a.	Yellow area

Explanation of Plate LXXXII.

All figures except figure 16 magnified × 6.

(For periods of forecast of emergence refer to Table II.)

Fig. 1. Nearly full grown female lac test, yellow spot on the posterior side below the anal tubercular opening.

„ 2. Full grown female lac test showing growth in the deep yellow spot towards the bottom of the posterior side of the test.

„ 3. Full grown female lac test showing the deep yellow area spread nearly to the middle of the posterior side.

Fig. 4. Full grown female lac test showing yellow area spread almost to the bottom of the posterior side. The bright portion of the yellow area immediately below the anal tubercular opening shows that the wax filaments are being deposited by the perivaginal glands and deposition of eggs has commenced.

„ 4*a*. Full grown female lac test of fig. 4 stage opened from the posterior side to show the mother insect, wax filaments deposited by the perivaginal glands and the deposition of eggs by the mother.

„ 5. Full grown female lac test showing the growth of the yellow area practically all over the posterior side behind the anal tubercular opening. The lightness in the colour all over the area indicates the presence of eggs and larvae in the test. If carefully observed the larvae can be seen moving within the test in this and the subsequent stages (figs. 6-9).

„ 6. Full grown female lac test shows the bright yellow area upto the anterior end of the anal tubercular opening on either side. If in this stage the yellow area on either side of the anal tubercular opening becomes in a straight line dorsally, it shows that without further increase in the yellow area the emergence of larvae will take place within 1-2 days.

„ 7. Full grown female lac test shows the growth of the bright yellow area advanced to midway between the anal tubercular opening and the brachial openings and also that the bright yellow area is in a straight line on either side of the test dorsally. Anal tubercle is partially drawn in.

„ 8. Full grown female lac test, bright yellow area as in fig. 7, but the anal tubercle completely drawn into the test. In this stage the larvæ can be seen inside the test through the anal tubercular opening.

„ 9. Full grown female lac test, bright yellow area as in figs. 7 and 8, but the larva coming out of the test through the anal tubercular opening. This means the emergence of larvae has started in the crop.

„ 9*a*. Full grown female lac test of fig. 9 stage opened from the posterior side, shows the test full of eggs and larvae and the anal tubercle displaced from its usual position.

„ 10. Full grown female lac test shows the bright yellow area on either side of the test in a line with the brachial openings indicating thereby that the emergence of larvae from the test has ceased. In certain cells the yellow area on either side of the test may develop slightly further laterally.

„ 10*a*. Female lac test of fig. 10 stage opened from the posterior side, showing the shrivelled mother lac insect forming a sort of lining to the anterior half of the test, the empty egg cases, wax filaments, some dead eggs and larvae, and a few living larvae which have failed to come out of the test.

„ 11. A nearly full grown female lac test in the early stage of parasitisation. The yellow spots in other parts of the test in addition to the one below the anal tubercular opening indicate presence of young parasite grubs in the lac cell.

„ 12. A chalcid larva to aid recognition of the grubs as one would generally find within the parasitised cells.

„ 13. A female lac test in which the mother insect has been eaten by parasite grubs except for its skin. The pale yellow colour of the test all over indicates the presence of mature parasitic larvae and pupae in the body of the lac insect.

Fig. 14. A full grown *S. trijuga* (*kusum*) female lac test showing the shape of isolated *kusum* cells and the yellow spot of the stage of fig 3.

„ 15. A full grown female lac test of yellow variety of lac insects as found in Jodhpur on Ber (*Z. Jujaba*) and in Kashmere on Khair (A. *catechu*), etc., showing the yellow area of the stage of fig. 4.

„ 16. A continuous incrustation on *kusum* (*S. trijuga*) twig, showing the full grown lac tests of different stages (figs. 1-8)×4.

Explanation of Plate LXXXIII.

Diagrammatic figures illustrating the changes in the shape of the lac insect and its muscles, due to contraction of muscles at the time of oviposition.

Fig. 17. Lateral view of the female lac test of the stage of fig. 1, showing the vacant space between the lac test and the female insect below the anal tubercular opening. This vacant space makes the test look deep yellow below the anal tubercle in fig. 1.

„ 17*a*. Lateral view of the female of fig. 17 stage, showing the arrangement of the tergo-sternal, sternal and tergal muscles in a circular shaped female before the muscles contract for oviposition.

„ 18. Lateral view of the female lac test of the stage of fig. 2, showing the increase in the vacant space and the shape of the female after the first contraction of tergo-sternal muscles.

„ 18*a*. Lateral view of the female of fig. 18 stage, showing depressions medially on the posterior and ventral side of the insect due to contraction of muscles.

„ 19. Lateral view of the female lac test of the stage of fig. 3, showing the increase in the vacant space and the shape of the female after the second contraction of the tergo-sternal muscles.

„ 19*a*. Lateral view of the female of fig. 19 stage showing the deepening of the depressions medially in the posterior and ventral side of the insect, due to contraction of muscles, and also the change in the shape of the muscle due to contraction.

„ 20. Lateral view of the lac test of the stage of fig. 4, showing the increase in the vacant space and the shape of the female after the third contraction of tergo-sternal muscles.

„ 20*a*. Lateral view of the female of fig. 20 stage, showing the deepening of the depression medially on the posterior and ventral side and change in the shape of the muscles, due to further contraction.

„ 21 Lateral view of the female lac test of the stage of fig. 5, showing increase in the vacant space and the shape of female after the fourth contraction of the tergo-sternal muscles.

„ 21*a*. Lateral view of the female of fig. 21 stage, showing that the depressions on the ventral and posterior side of the insect medially have come to lie more or less in a straight line, and also the change in the shape of the muscles. The dotted line in this and the next two figures shows the position of the lateral part of the body in that region.

„ 22. Lateral view of the female lac test of the stage of fig. 7, showing increase in space and shape of the female after the sixth contraction of muscles.

Fig. 22*a*. Lateral view of the female of fig. 22 stage showing further change in shape of the body medially and the change in the shape of muscles.

„ 23. Lateral view of the female lac test of the stage of fig. 9, showing increase in space and the shape of female after the seventh contraction of muscles.

„ 23*a*. Lateral view of the female of fig. 23 stage showing the change in the shape of body and muscles.

„ 24. Lateral view of the female lac test of the stage of fig. 10 showing that the insect forms only a sort of lining to the anterior half of the test.

„ 24*a*. Lateral view of the female of fig. 24 stage showing the final change in the shape of the body of the female and its muscles.

„ 25. Diagrammatic representation of the muscles and perivaginal glands of the right half of the body.

„ 25*a*. Honeycombed pores of an individual perivaginal gland cluster, seen from the ventral side in a cleared specimen.

„ 25*b*. Tergal muscles in the anal tubercle and between it and the pedicel of dorsal spine.

„ 26. Lateral view of the attachment of tergo-sternal, sternal and tergal muscles in a female lac insect of pyriform shape.

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STUDIES IN THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE IN SOME LEGUMINOUS CROP PLANTS OF INDIA.

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(With Plates LXXXIV-XCI)

Investigations on the agronomy and genetics of most of the leguminous crop plants of India have been undertaken since the beginning of this century and results of great economic importance have been obtained in many cases. The morphology of these plants, however, has received scant attention. The study of the morphology of crop plants has, in many cases, given results of great economic importance. A study of the morphology of the reproductive organs in particular, enables us to determine the nature and extent of sterility in plants and helps us considerably in controlling seed production.

The present investigation was undertaken primarily with the object of working out of the development of the ovule and embryosac in some of the leguminous crop plants in India of which nothing is known at present.

Literature.

Guignard [1881] published his paper on the morphology of about 40 species of the Leguminosae. His account shows that a single archesporial cell is most common in the group; the archesporial cell on division gives rise to a primary parietal cell and a megaspore mother cell; the former may remain undivided or produce a tissue varying in amount; the megaspore mother cell may directly develop into the embryosac, or may give rise to a row of 2, 3 or 4 megaspores, of which the chalazal or the one next to it may function; the mature embryosac is usually eight-nucleate. The antipodals persist longer in Caesalpinoideae and Mimosioideae than in Papilionoideae; the fusion of the polar nuclei may occur in the centre of the embryosac, against the inner wall or in contact with the egg apparatus.

Saxton [1907] described the embryosac of *Cassia tomentosa* and found a deeply buried megaspore mother cell; an usual tetrad of megaspores is produced of which the innermost one (chalazal) functions; the polars fuse early and the antipodals are persistent and form an absorptive tissue.

Martin [1914] published a paper on the comparative morphology of *Trifolium pratense, T. hybridum, T. repens, Medicago sativa, and Vicia americana.* He states that the ovules are campylotropous with two integuments, the outer one preceding the inner; a multiple archesporium is common; one parietal cell is cut off which gives rise to the parietal tissue; in *M. sativa* the megaspore mother cells were found in a few cases without parietal cells; one or more longitudinal rows of four megaspores are produced of which the lowest one functions. During the development of the embryosac much of the nucellar tissue is digested and absorbed and as a result the mature embryosac comes to lie directly against the inner integument; the antipodals are ephemeral.

Brown [1917] studied five varieties of *Phaseolus vulgaris.* She states that there is an axial row of only three megaspores, the outer cell resulting from the heterotypic division not functioning. Her work was confirmed by Weinstein [1926].

Reeves [1930] reinvestigated the development of the embryosac in *Medicago sativa.* He states that the archesporium consists of one or more cells; a parietal cell is cut off but the amount of parietal tissue produced is variable; several tetrads may be found, but not more than one mature embryosac has been recorded; the mature embryosac is usually eight-nucleate. The antipodals degenerate very early but the polars remain partially fused before fertilization, and the embryosac grows at the expense of the adjacent tissue.

Maheshwari [1931] worked on *Albezzia lebbek.* He states that the archesporium may consist of one or more cells; the parietal cell by periclinal and anticlinal divisions produces a heavy wall; of the four megaspores the chalazal one always functions; the polars do not fuse before fertilization and the antipodals are persistent till fertilization.

Material and methods.

The material used in this investigation was obtained from plants grown in the university experimental garden, from seeds obtained from the Principal, Agricultural College, Coimbatore. The development of the embryosac in the following plants was investigated:—

(1) *Pachyrhizus angulatus* Rich.
(2) *Cajanus indicus* Spreng.
(3) *Dolichos Lablab* Linn.
(4) *Pisum sativum* Linn.
(5) *Lathyrus sativus* Linn.

The material was fixed in Allen's modification of Bouin's fluid, and in Licent's fixative. To remove the waxy coating the material was first dipped for a very

short time in acetic alcohol (1 : 2) and then immersed in the fixatives. This procedure gave satisfactory result. The fixation was carried out between 9 a.m. and 1 p.m. in the field; the material fixed between 10 and 11 a.m. showed numerous divisional stages. In *Pachyrhizus*, *Cajanus* and *Dolichos* the dense mat of hairs on the ovary had to be removed by scraping. The material was left for 24 hours in the fixing fluid. It was dehydrated, cleared and infiltrated in the usual way.

Sections were cut 6 to 10 microns thick, depending on the age of the material and the stage required for study.

Heidenhain's iron alum haematoxylin was chiefly used for staining; a number of preparations were counter-stained with orange G. The sections were mounted in Canada balsam.

Ovule.

As is characteristic of the Leguminosae, the ovules are borne in two rows, one on each side of the ventral suture. At first, the ovule is orthotropous, but when it comes in contact with the dorsal wall of the ovary the apex of the ovule curves and ultimately the ovule becomes campylotropous to half-anatropus. It rarely becomes strictly anatropus.

The integuments of the ovule in all the plants studied are two in number; in *Dolichos*, the inner integument always develops first; in *Pachyrhizus* and *Pisum* the integuments appear almost simultaneously but in *Cajanus* and *Lathyrus* the outer integument precedes the inner. The outer integument always grows faster and soon overtakes the inner. The inner integument is mostly composed of two layers of cells except near the micropyle where it is thicker in some species. The outer integument is variable in thickness in the different species, but it is always thicker than the inner integument, and more so at the micropylar end. In *Cajanus*, *Dolichos*, *Pisum* and *Lathyrus* (Plate XCI) both the integuments take part in the formation of the micropyle; in the first two species, however, only that portion of the inner integuments which is towards the funiculus takes part in the process; in *Pachyrhizus*, however, the outer integument only takes part in the formation of the micropyle.

Embryosac.

As the process of embryosac development was found to be similar in all the species investigated, a general description of the development of the female gametophyte is given below and the points of differences indicated.

The ovule first appears as a blunt papillate process and consists of a group of homogenous cells. Very soon one or more of the nucellar cells become differentiated from the rest. In *Pachyrhizus* usually a group of archesporial cells differentiates

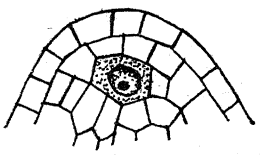


Fig. 1.

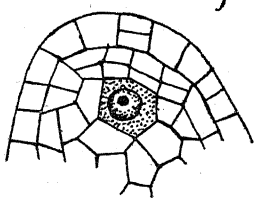


Fig. 2.

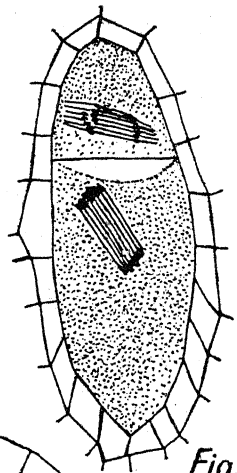


Fig. 5.

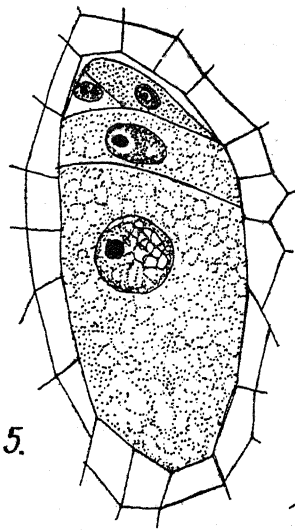


Fig. 6.

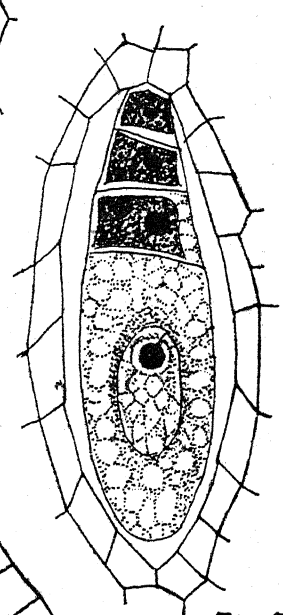


Fig. 7.

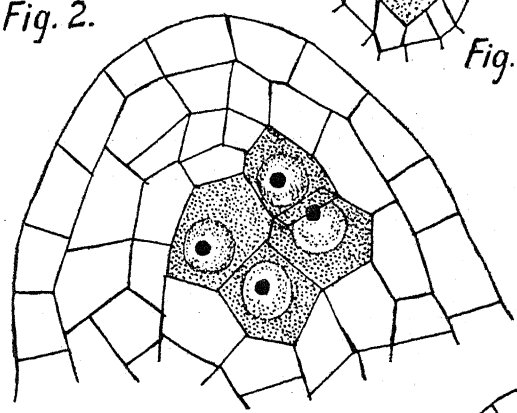


Fig. 3.

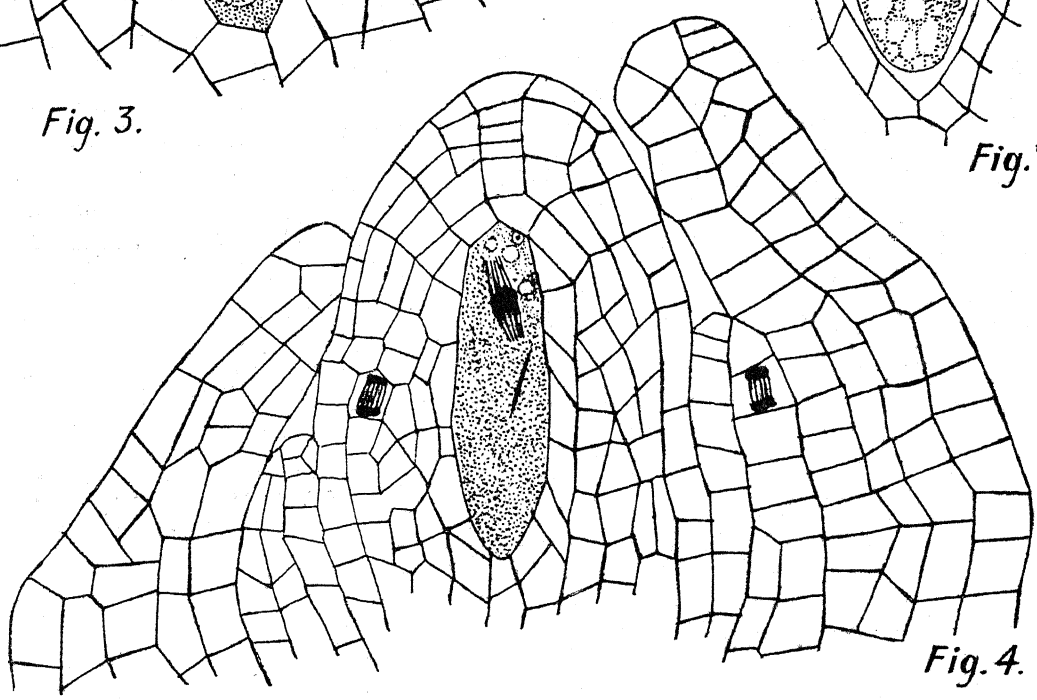


Fig. 4.

Figs. 1-7. *Pachyrhizus angulatus* Rich.

Fig. 1. The origin of an archesporial cell (×500). Fig. 2. An archesporial cell with division of its covering cells (×500). Fig. 3. A group of four archesporial cells (×500). Fig. 4. Reduction division of the megaspore mother cell (×800). Fig. 5. Homoetypic division (×1100). Fig. 6. A tetrad of megaspores (×1100). 7. A tetrad of megaspores; the three upper cells have disintegrated (×1100).

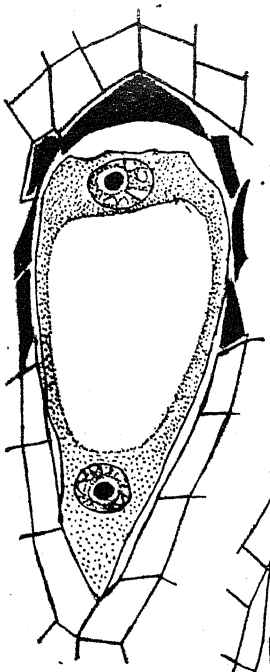


Fig. 8

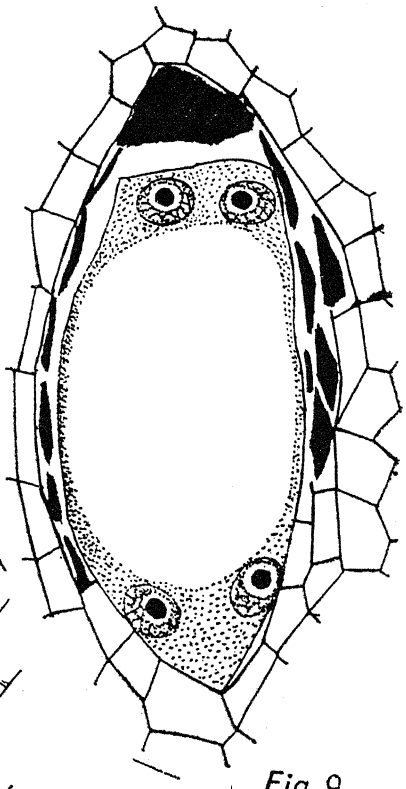


Fig. 9.

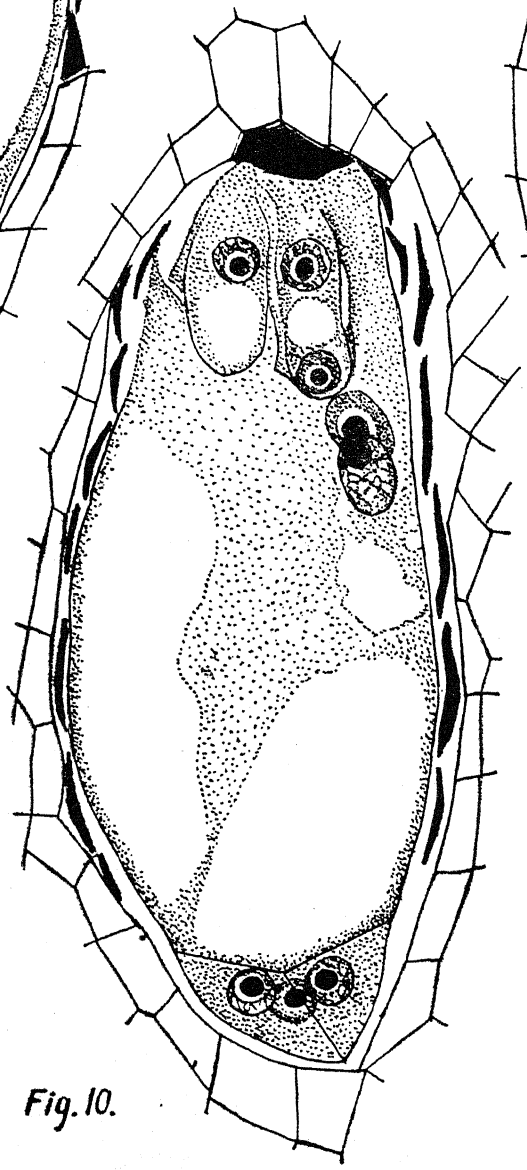


Fig. 10.

Fig. 12

Fig. 13.

Fig. 14.

Fig. 15.

Figs. 8-10 and 12-15. *Pachyrhizus angulatus.*

Fig. 8. A binucleate embryosac (×1100). Fig. 9. A four-nucleate embryosac (×1100). Fig. 10. An advanced eight-nucleate embryosac showing the synergids, the egg, the fusion of polar nuclei and the antipodals (×1100). Fig. 12. Chromosome number of *Pachyrhizus angulatus* Rich. (×1100). Fig. 13. Chromosome number of *Cajanus indicus* Spreng. (×1100). Fig. 14. Chromosome number of *Pisum sativum* Linn. (×1100). Fig. 15. Chromosome number of *Lathyrus sativus* Linn. (×1100).

in the third layer of the nucellar tissue and sometimes have a linear arrangement (Plate LXXXIV, fig. 3). A single archesporial cell has also been observed in a few cases in this material (Figs. 1, 2). In *Cajanus*, a single archesporial cell is usually recognised at the third layer of the nucellar tissue (Plate LXXXV. fig. 16), but in some preparations a number of archesporial cells have been noted, and in one flower all the young ovules were found to contain two archesporial cells. In *Dolichos* also a single archesporial cell is commonly met with at the third layer of the nucellar tissue (Fig. 26) but two equally differentiated archesporial cells have also been noted. In *Lathyrus* usually a single hypodermal cell is differentiated out as the archesporial cell early in the development of the ovule (Fig. 45) but multiple archesporium is also noted. In *Pisum* all the preparations showed the megaspore mother cell situated at the third layer of the nucellar tissue, and, considering the size and the orientation of the surrounding cells, it appears that the archesporial cell here too differentiates at the third layer and acts directly as the megaspore mother cell (Fig. 35).

In *Pachyrhizus*, *Cajanus*, *Dolichos* and *Pisum* the archesporial cell directly functions as the megaspore mother cell and does not cut off any parietal cell as has been noted in a number of leguminous and other plants by various investigators. The megaspore mother cell is therefore found in the third layer of the nucellar tissue capped generally by two layers of nucellar cells. In *Pachyrhizus*, these cover cells divide both periclinally and anticlinally and as a result the megaspore mother cell is pushed inside and becomes deeply located in the nucellar tissue (Fig. 2). Division of the cover cells seldom occurs in *Dolichos* and in *Cajanus*. In *Lathyrus* the archesporial cell divides to give rise to a megaspore mother cell and a primary parietal cell; the parietal cell divides both periclinally and anticlinally but the periclinal division very seldom takes place, so that, in the majority of cases the megaspore mother cell lies two cell layers deep. In *Pisum* as in *Pachyrhizus* the megaspore mother cell is pushed inside the cells of the nucellus by the division of the nucellar cells above (Fig. 36).

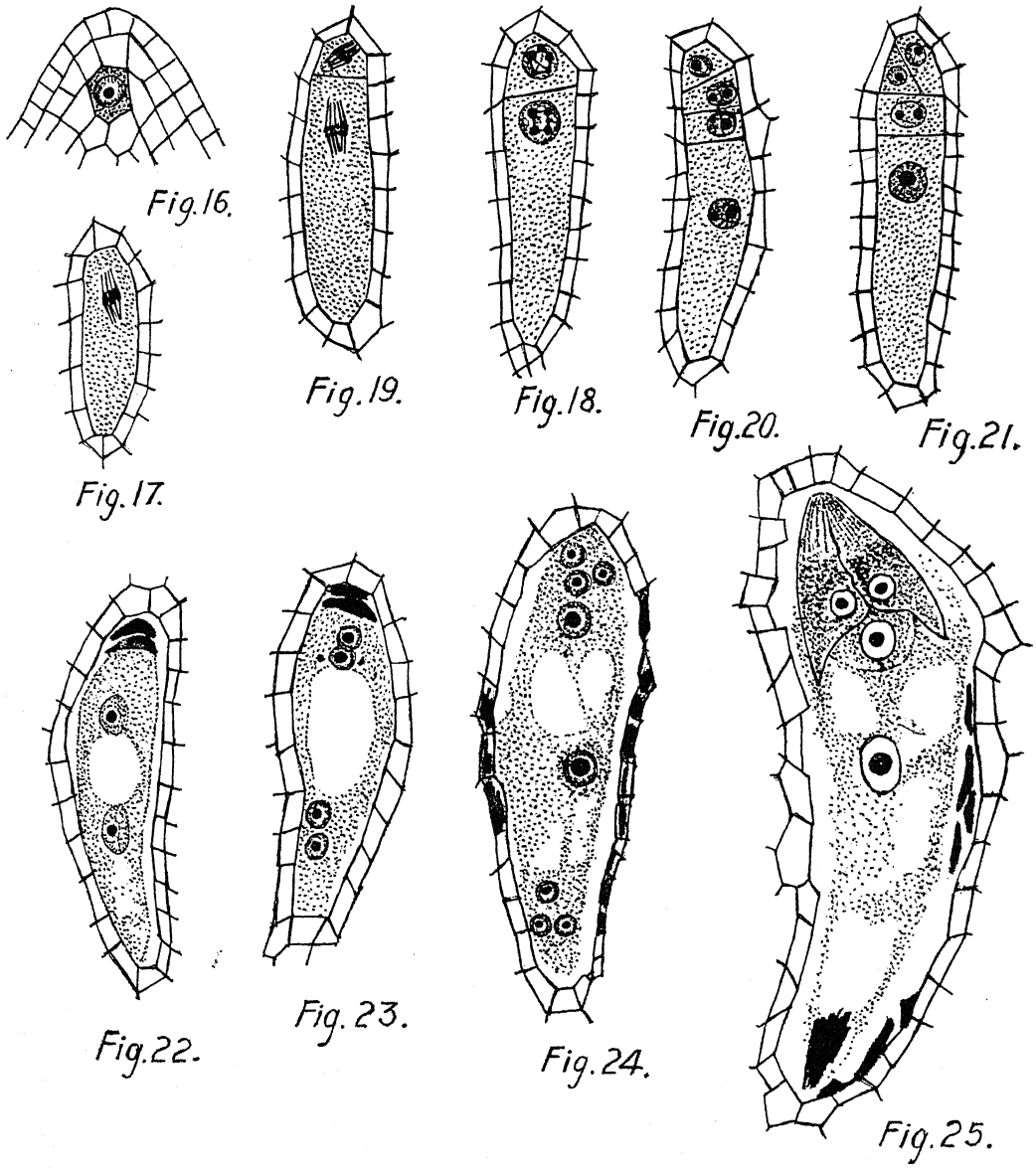
The megaspore mother cell after its differentiation can be easily detected by its granular cytoplasm, bigger nucleus and greater chromaticity. The cytoplasm in most cases appears to be somewhat granular and fills the entire cell. The development of the pollen mother cells in the anthers is well-advanced at this stage of the megaspore mother cell, and, in most cases, the second division has been completed and pollen quartets have been formed. The megaspore mother cell increases in size and undergoes the usual changes of the heterotypic prophase and at metaphase a well defined spindle is noted in every case (Figs. 4, 17, 28, 37, 47). The heterotypic spindle in all cases has been observed to be at the top of the cell and is oriented obliquely. As in most other plants the spindle is seen to be composed of two types

of fibres; the thicker strands of fibres are interspersed between the thinner ones and appear to be connected with the chromosomes and presumably help them in their anaphasic movement. The dual nature of the bivalents while arranged in the heterotypic spindle could be made out in every case. This is particularly evident when the anaphasic movement is initiated. The movement to the poles appears to be quite regular and in no case were "non disjunction" or other irregularities observed. On reaching the poles the chromosomes generally clump together. A well defined interphase follows. A cell plate is formed at about this time and divides the cell into two halves of which the upper one in every case is smaller than the lower.

Comparative measurements of the upper and lower cells of the dyad are given below:—

	Upper cell	Lower cell
1. *Pachyrhizus*	8μ	40μ
2. *Cajanus*	12μ	60μ
3. *Dolichos*	16μ	68μ
4. *Pisum*	8μ	40μ
5. *Lathyrus*	16μ	40μ

The heterotypic division is quickly followed by the homoetypic division. The arrangement of the spindle is rather variable. The lower cell (chalazal) in *Pachyrhizus*, *Dolichos*, and *Pisum* shows generally a more advanced stage in mitosis than the upper (Figs. 5, 29, 38). In *Lathyrus* this is so very pronounced, that when the lower cell has divided and formed two megaspores, the upper cell is still in the divisional stage (Fig. 49). In *Cajanus* both the cells appear to divide simultaneously (Fig. 19). The planes of division of the dyads appear to be variable. Generally the dyads are divided by periclinal walls. In *Pachyrhizus*, *Cajanus*, *Dolichos* and *Pisum*, the upper cell of the dyad is sometimes divided by a half anticlinal (oblique) to a strictly anticlinal wall (Figs. 6, 21, 30, 39). In *Lathyrus*, however, the micropylar cell of the dyad always divides by an anticlinal wall, thus suggesting a faint resemblance between microspore and megaspore tetrads (Figs. 50, 51). As a result of these two divisions a tetrad of megaspores is produced in every case (Figs. 6, 30, 39, 50). The lowest or the chalazal megaspore alone functions while the rest degenerate. In *Pachyrhizus* and *Dolichos*, the upper megaspores degenerate first; in *Lathyrus*, however, the megaspore just above the functioning one is the first to show signs of degeneration (Fig. 51).



Figs. 16-25. *Cajanus indicus* Spreng.

Fig. 16. The origin of an archesporial cell of *Cajanus*. Fig. 17. Reduction division of the megaspore mother cell. Fig. 18. A Dyad. Fig. 19. Homoetypic division. Figs. 20, 21. Tetrad of megaspores. Fig. 22. A binucleate embryosac. Fig. 23. A four-nucleate embryosac. Fig. 24. An eight-nucleate embryosac note migration of polar nuclei. Fig. 25. A mature embryosac; note the fileform apparatus; antipodals have disintegrated.

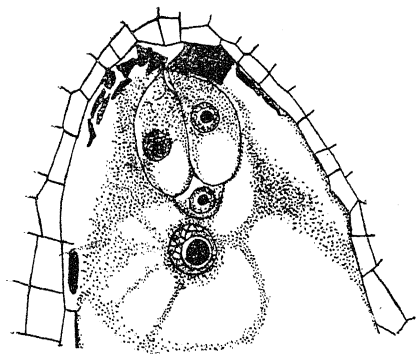


Fig. 11.

Fig. 11. A portion of the mature embryosac of Pachyrhizus showing the egg apparatus and primary endosperm nucleus. (×1100.)

The nucleus of the embroysac increases in size before it commences its activity. It divides, and the daughter nuclei migrate to opposite poles of the embryosac; a central vacuole is noted at this stage (Figs. 8, 22, 32, 52). The embryosac generally increases in size before the four-nucleate stage is reached. As is usual at this stage the nuclei are distributed equally at the two poles of the embryosac (Plate LXXXV, figs. 9 and figs. 23, 33, 42, 54). By the division of these four nuclei the eight-nucleate stage is reached and the embryosac increases much in size. Three nuclei at the chalazal end and three nuclei at the micropylar end of the embryosac become specialized from the general cytoplasm of the embryosac by a well-developed " hautschicht " round each of them. The three micropylar cells organise the egg-apparatus and the three chalazal ones, the antipodals. The remaining two nuclei, devoid of any such membrance, migrate towards each other and meet near the micropylar end, in close vicinity of the egg to form the primary endosperm nucleus. This is particularly noticeable in *Pachyrhizus*, and *Cajanus* (Plate LXXXVI, figs. 11, and fig. 25.) The antipodals in *Pachyrhizus* are relatively persistent and degenerate only after, and not before, polar fusion. The two polar nuclei in *Dolichos*, *Pisum* and *Lathyrus* do not fuse even when the antipodals have degenerated but lie side by side, or one above the other near the centre of the embryosac, and most probably remain in this stage till fertilization (Figs. 34, 44, 55).

The egg apparatus consists of two synergids, vacuolate at the base, with the nucleus at the apical end, and an egg, vacuolate at the apex, with the nucleus at the base. The synergids are more or less beaked in all the species. In *Cajanus* a well developed filiform apparatus of each synergid was noted (Plate LXXXVII, fig. 25). In the other species investigated the filiform apparatus could not be detected.

The digestion and absorption of nucellar tissue during the growth of the embryosac is an interesting feature in this family. In *Pachyrhizus*, *Cajanus*, *Lathyrus* and *Pisum* the first sign of degeneration and absorption of the tissue is noticed when the embryosac has reached the binucleate stage. In *Dolichos*, however, no such sign was found at the binucleate stage and it is only apparent when the embryosac has reached the four-nucleate stage (Plate LXXXVII, fig. 33).

The final position of the embryosac is determined by the degree of absorption of the nucellar tissue. In *Pachyrhizus* in spite of much absorption of the nucellar tissue, the mature embryosac is surrounded and capped by several layers of nucellar cells and as such the mature embryosac is not in direct contact with the micropyle or the inner integument. In *Dolichos* and in *Cajanus*, though there is much degeneration of the nucellar tissue, yet the mature embryosac is usually capped by two cell layers in the former and by only one cell layer in the latter. In *Pisum*

and *Lathyrus* the degeneration of the nucellar tissue extends up to the epidermal layer so that the mature embryosac in these species is in direct contact with the inner integument and the micropyle.

Sterility.

No evidence of wide spread sterility of the female gametophyte was noted in any of the species investigated. Only occasional degeneration of the megaspore mother cell or of the embryosac in the binucleate or 4-nucleate stage was met with. Hence sterility of the female gametophyte cannot be considered as a factor of any importance in affecting seed production in these plants.

Chromosome number.

The chormosome numbers of plants belonging to the family Leguminosae have been investigated by a number of investigators and many new numbers have been recorded in recent years. The lowest haploid number as yet determined in this family is six and is found in certain species of *Vicia*. It is interesting to note that other species of *Vicia* have been found with seven or twelve haploid chormosomes. Of the leguminous crop plants Rau [1929] has determined 24 diploid chromosomes for *Vigna catjang*, *Dolichos Lablab*, *Phaseolus radiatus* and *Phaseolus mungo*, and fourteen ($2n$) in *Cicer arietinum*. Seven haploid chomosomes have been recorded in *Lens esculentum* by Sakamura [1920]. The same number has been recorded in *Pisum arvense* by a number of workers.

In the present investigation an attempt was made to determine the chromosome numbers of the plants studied. The computation was made from the meiotic divisions of microspore mother cells. It was found that *Pachyrhizus angulatus* and *Cajanus indicus* both possess eleven haploid chromosomes (Figs. 12, 13). *Lathyrus sativus* showed seven chromosomes in the meiotic divisions (Fig. 15). The same number has been recorded by Latter [1926] in *L. odoratus*. *Pisum sativum* also showed seven haploid chromosomes which agrees with the counts made by previous investigators (Fig. 14). Satisfactory evidence as to the choromosome number of *Dolichos Lablab* was not obtained.

Discussion.

The data obtained in course of this investigation go much in support of our previous stock of knowledge in this subject.

The flowers, as characteristic of the leguminosæ, are monocarpellary. In *Dolichos* a flower was found to possess two carpels with separate styles and stigmas but conjoined ovaries. This is of course an abnormal flower and such instances are very rare.

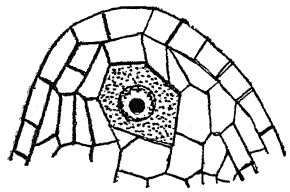


Fig. 26

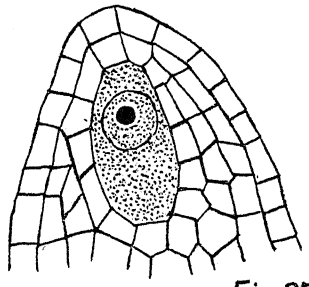


Fig. 27.

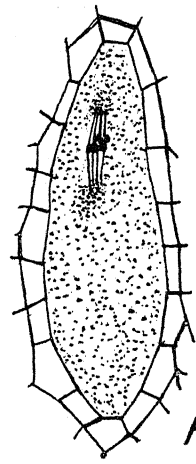


Fig. 28.

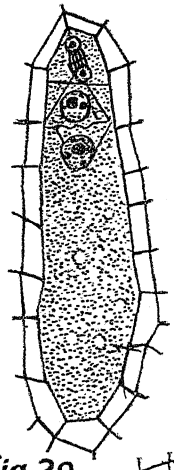


Fig. 29.

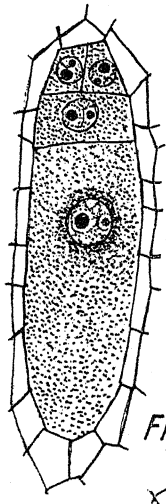


Fig. 30.

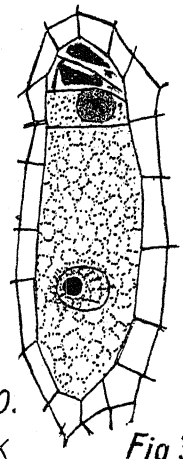


Fig. 31.

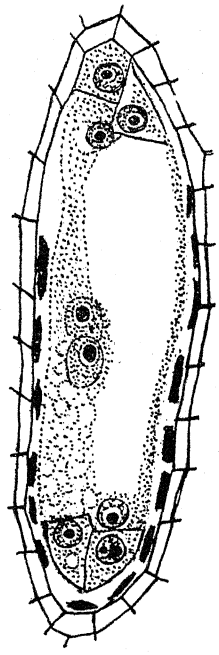


Fig. 34.

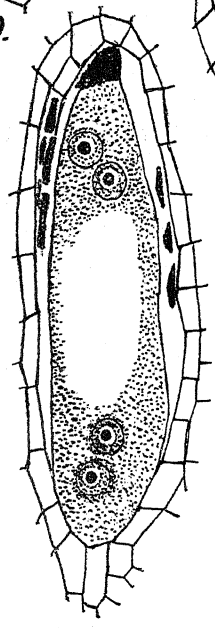


Fig 33

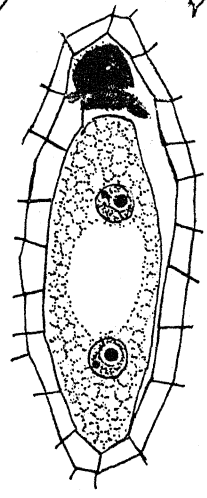
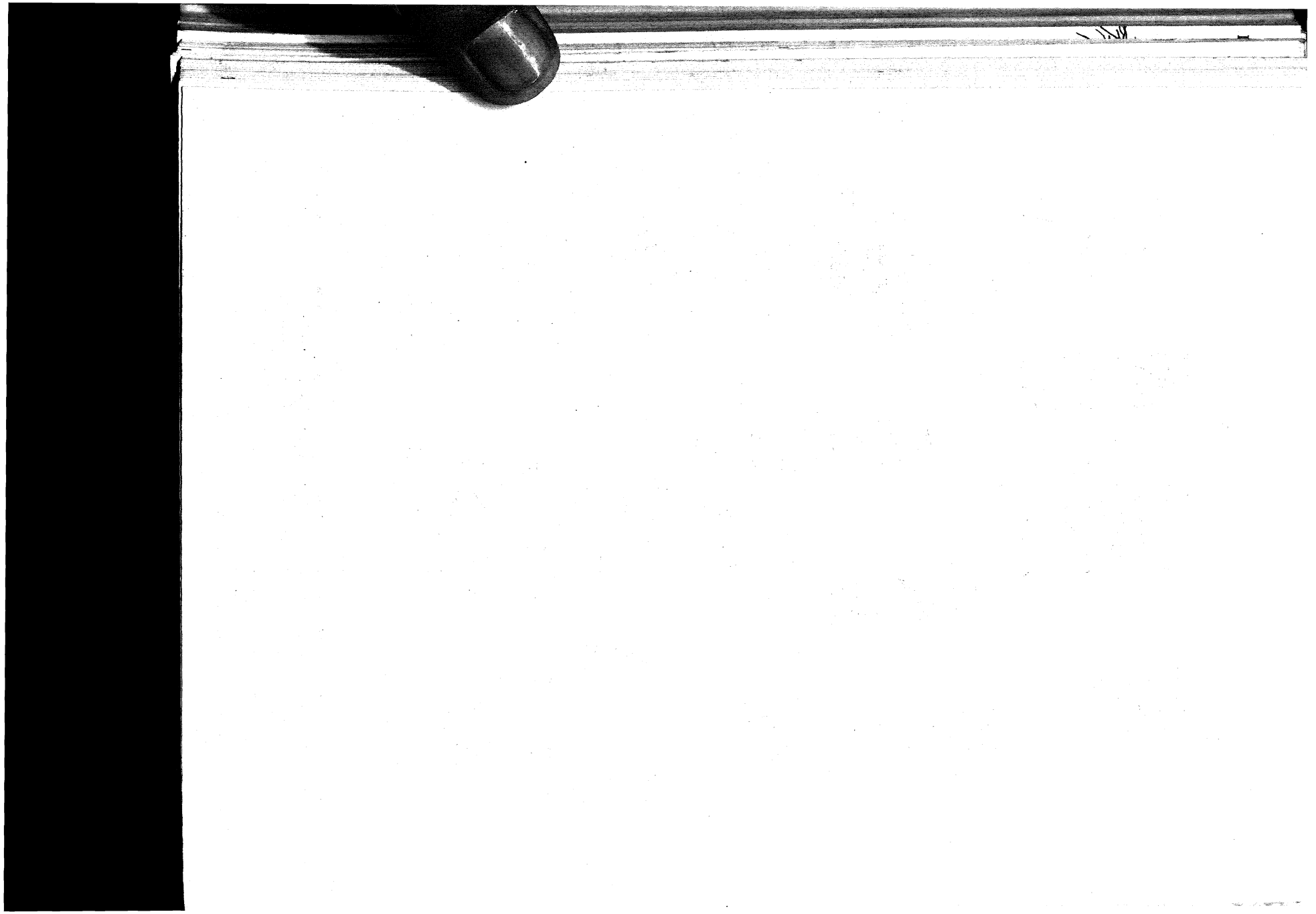
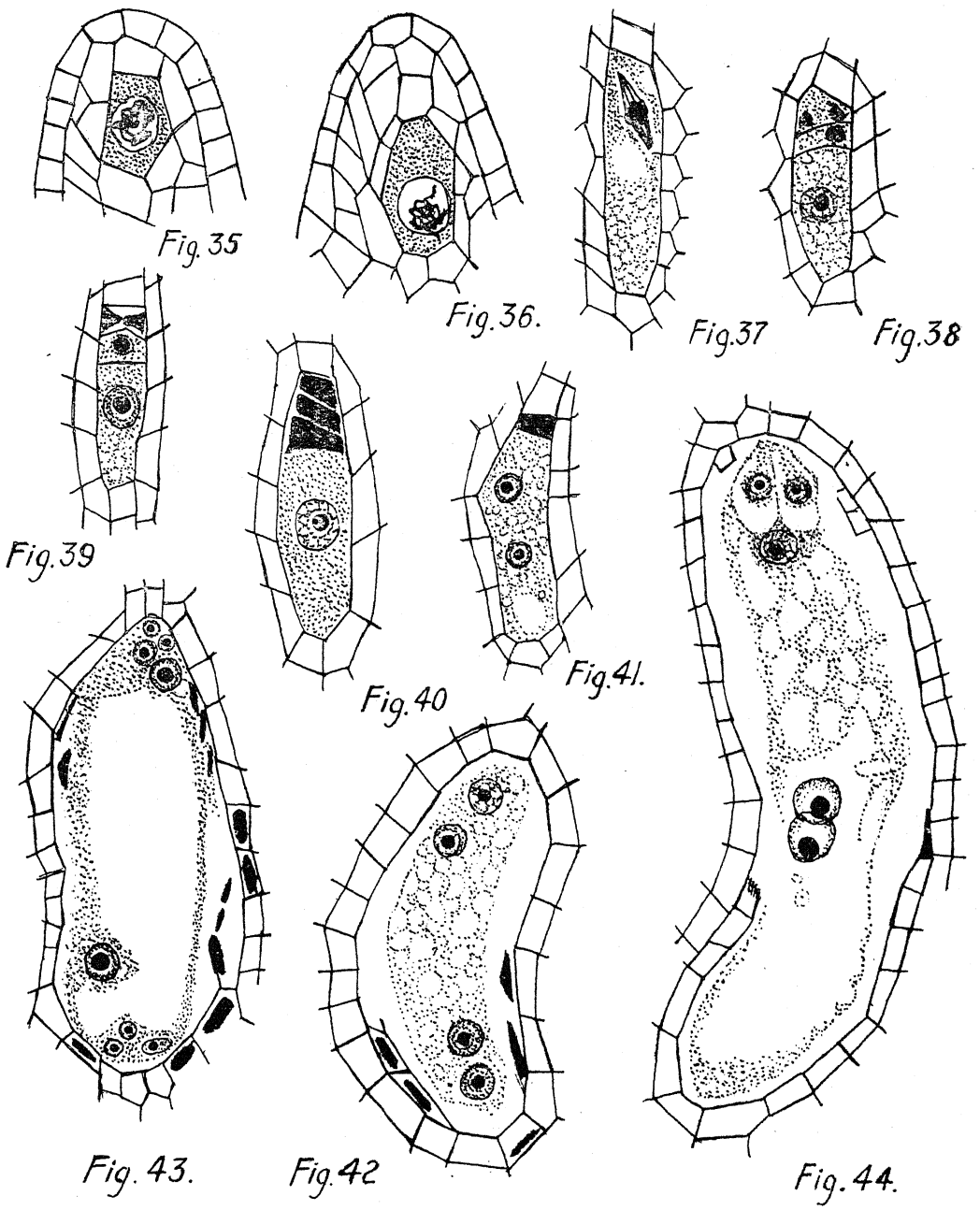


Fig. 32.

Figs. 26-34. *Dolichos Lablab* Linn.

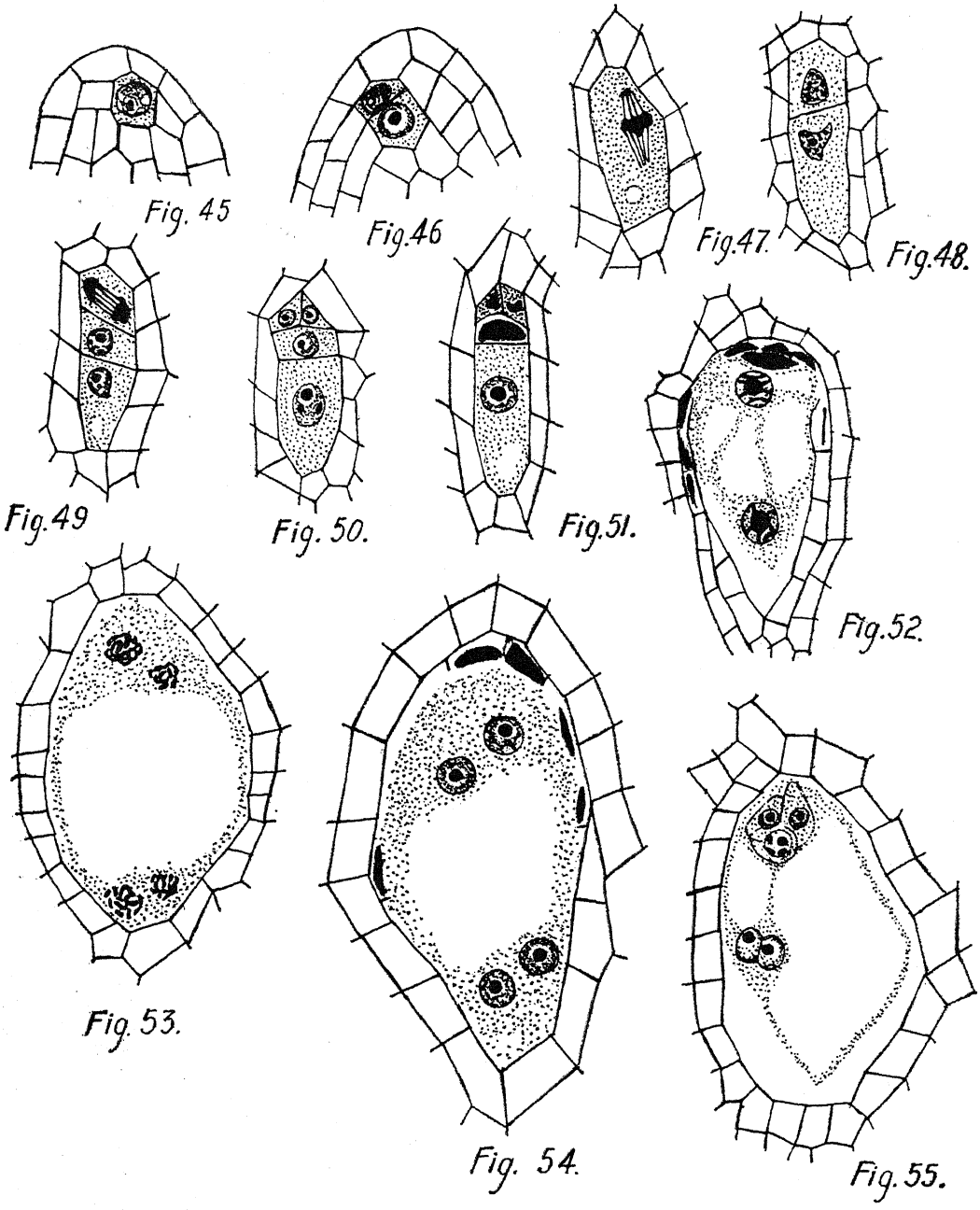
Fig. 26 The origin of an archesporial cell. Fig. 27. Megaspore mother cell. Fig. 28. Reduction division of the megaspore mother cell. Fig. 29. Homœotypic division of megaspore mother cell. Fig. 30. A tetrad of megaspores. Fig. 31. Disintegration of the three spores of a tetrad. Fig. 32. A binucleate embroysac, Fig. 33. A four-nucleate embryosac. Fig. 34. A mature embryosac. (×500).





Figs. 35-44. *Pisum sativum* **Linn.**

Fig. 35. A megaspore mother cell in synezesis. **Fig. 36.** A megaspore mother cell in synezesis; the flattening of the nucleolus to be noticed. **Fig. 37.** Reduction division. **Fig. 38.** Homoetypic division. **Figs. 39, 40.** Disintegration in tetrads. **Fig. 41.** A binucleate embryosac. **Fig. 42.** A four-nucleate embryosac. **Fig. 43.** An eight-nucleate embryosac; migration of the polar nuclei to be noticed. **Fig. 44.** A mature embryosac showing the egg apparatus and polar nuclei. (×500).



Figs. 45-55. *Lathyrus sativus* Linn.

Fig. 45. The origin of an archesporial cell. Fig. 46. An archesporial cell divided into a megaspore mother cell and a parietal cell. Fig. 47. Reduction division. Fig. 48. A dyad. Fig. 49. Homœtypic division. Fig. 50. A tetrad of megaspores. Fig. 51. Disintegration of the three megaspores in a tetrad. Fig. 52. A binucleate embryosac. Fig. 54. A four-nucleate embryosac. Fig. 55. A mature embryosac showing the egg apparatus and polar nuclei. (×500).

The ovules are more or less campylotropous in all the species investigated. Similar orientation of the ovule has been recorded by Martin [1914] in three species of *Trifolium*, in *Medicago sativa* and in *Vicia americana*.

The development of the integuments agrees with the records of previous investigators on this line. In *Dolichos*, the inner integument starts first, a fact well established in various other species of this family, by Guignard [1881], Reeves [1930], Maheshwari [1931] and others. In *Cajanus* and *Lathyrus* the outer integument precedes the inner and similar observations have been made by Martin [1914] in the species studied by him.

The inner integument is almost always composed of two layers of cells and this is also the opinion of previous investigators.

It is interesting to note that in *Pisum*, *Lathyrus*, *Dolichos* and *Cajanus* where the mature embryosac has digested and absorbed the cover cells completely or nearly so, both the integuments are found to take part in the formation of the micropyle; but in *Pachyrhizus* where the mature embryosac is covered by a heavy wall of nucellar cells, only the outer integument takes part in the formation of the micropyle. It is tempting to conclude from the above facts that the growth of the inner integument may be related to the degree of digestion and absorption of nucellar cells.

The archesporium, we find, usually consists of a group of cells in *Pachyrhizus* and shows multiple tendency in *Dolichos*, *Cajanus* and *Lathyrus*. Similar multiple archesporium has been recorded in *Vicia* and *Trifolium* by Martin [1914], in *Phaseolus* by Brown [1917] in *Medicago sativa* by Reeves [1930], and in *Albezzia lebbek* by Maheshwari [1931]. Multiple condition of archesporium may here be explained as the remnant of a primitive feature.

The archesporial cell cuts off a parietal cell in *Lathyrus* only (Plate XC), but in the remaining four species no parietal cell was observed. Absence of parietal cell has been recorded in *Lathyrus odoratus* by Jönsson [1879-80] in *Orobus angustifolius* by Guignard [1881], and in *Medicago sativa* by Martin [1914].

In *Cajanus* and *Dolichos* it has been observed that the absence of the parietal cell is usually followed by the absence of the division of the cover cells, but in *Pachyrhizus* and *Pisum*, inspite of the absence of the parietal cell, the cover cells divide actively and as a result the soprogenous tissue is more or less deeply imbeded. The situation is hard to explain, but it is certain that the presence or absence of the heavy layer of cover cells has some specific value and may have some relation with the growth of the embryosac and the process of fertilization.

The megaspore mother cell by two successive divisions gives rise to a tetrad of megsapores in all cases of which the chalazal one always functions. No abnormality in the number or behaviour of the megaspores was observed in the species

studied; hence it may be safely concluded that the embryosac development is of the normal type in all cases. It may be noted in this connection that Jönsson [1879-80] reported the embryosac development in *Lathyrus odoratus* to be of the "Scilla" type. Critical study of *Lathyrus sativus* was undertaken and it was found that the upper cell of the dyad as usual produced two of the megaspores.

The chalazal megaspore is always found to be the largest of the four, a condition apparently related to the fact that it is this one which functions. The remaining three megaspores always degenerate before the chalazal megaspore divides;

The nucleus of the embryosac by three successive free nuclear divisions gives rise to the eight nuclei of the mature embryosac — a general feature in angiosperms (Plate XCI). In one instance in *Dolichos*, however, a mature embryosac was found to contain one extra nucleus at the micropylar end, *i.e.*, the female gametophyte was nine-nucleate. No mature embryosac with less than eight nuclei was observed in the species studied.

The antipodals, as characteristic of the Papilionoideæ, are more or less ephemeral in all the species; this fact agrees with the observation of Guignard [1881].

The polars fuse completely in *Pachyrhizus* and *Cajanus* and the fusion nucleus lies close to the egg. Similar polar fusion before fertilization was observed by Guignard [1881] who remarks that in Leguminosæ the polar nuclei fuse before fertilization except in *Viceæ*. The polar nuclei in *Pisum*, *Dolichos* and *Lathyrus*, however, do not fuse in the mature embryosac. Similar cases have been recorded by Martin [1914] in all the species investigated by him and also by Maheshwari [1931] in *Albezzia lebbek*.

The synergids are of the usual structure, only those of *Cajanus* possess filiform apparatus. Filiform apparatus has been found in this family in *Trifolium pratense* by Martin [1914] and in *Albezzia lebbek* by Maheshwari [1931].

The embryosac grows at the expense of the adjacent tissue in all the species, a fact which has been well established by various investigators.

Summary.

The development of the female gametophyte was studied in the following leguminous crop plants:—*Pachyrhizus angulatus*, *Cajanus indicus*, *Dolichos Lablab*, *Pisum sativum* and *Lathyrus sativus*.

1. In all the species studied, the curvature of the ovules is towards the apex of the ovary. The ovules are more or less campylotropous.

2. A hypodermal cell or cells differentiate as the archesporium in *Lathyrus*; in the remaining four species the archesporium differentiates at the third layer of the nucellus and usually consists of a group of cells.

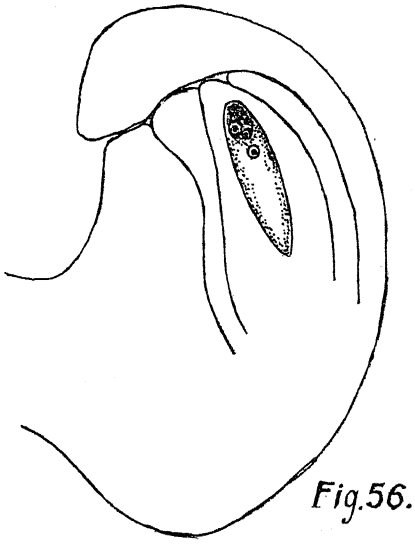


Fig. 56.

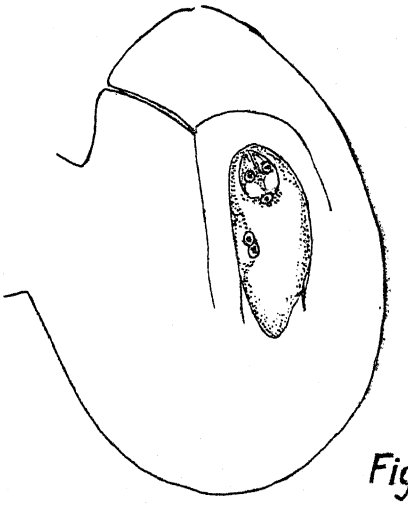


Fig. 57.

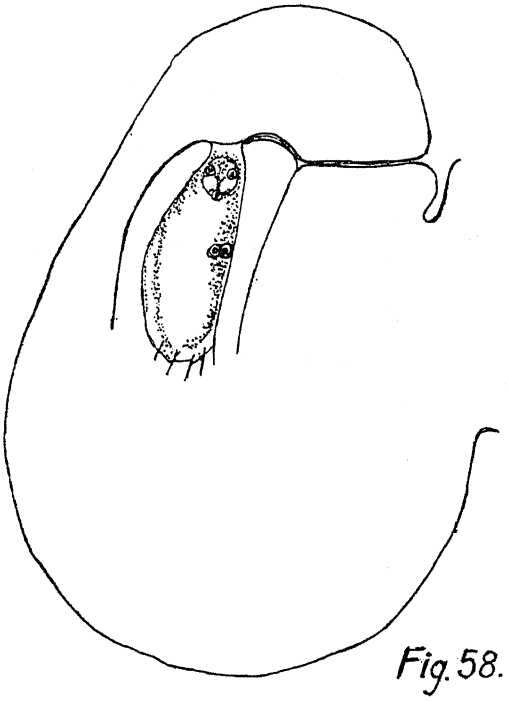


Fig. 58.

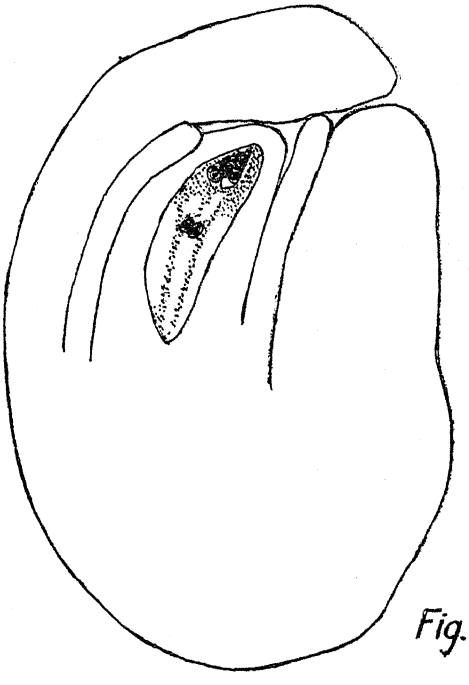
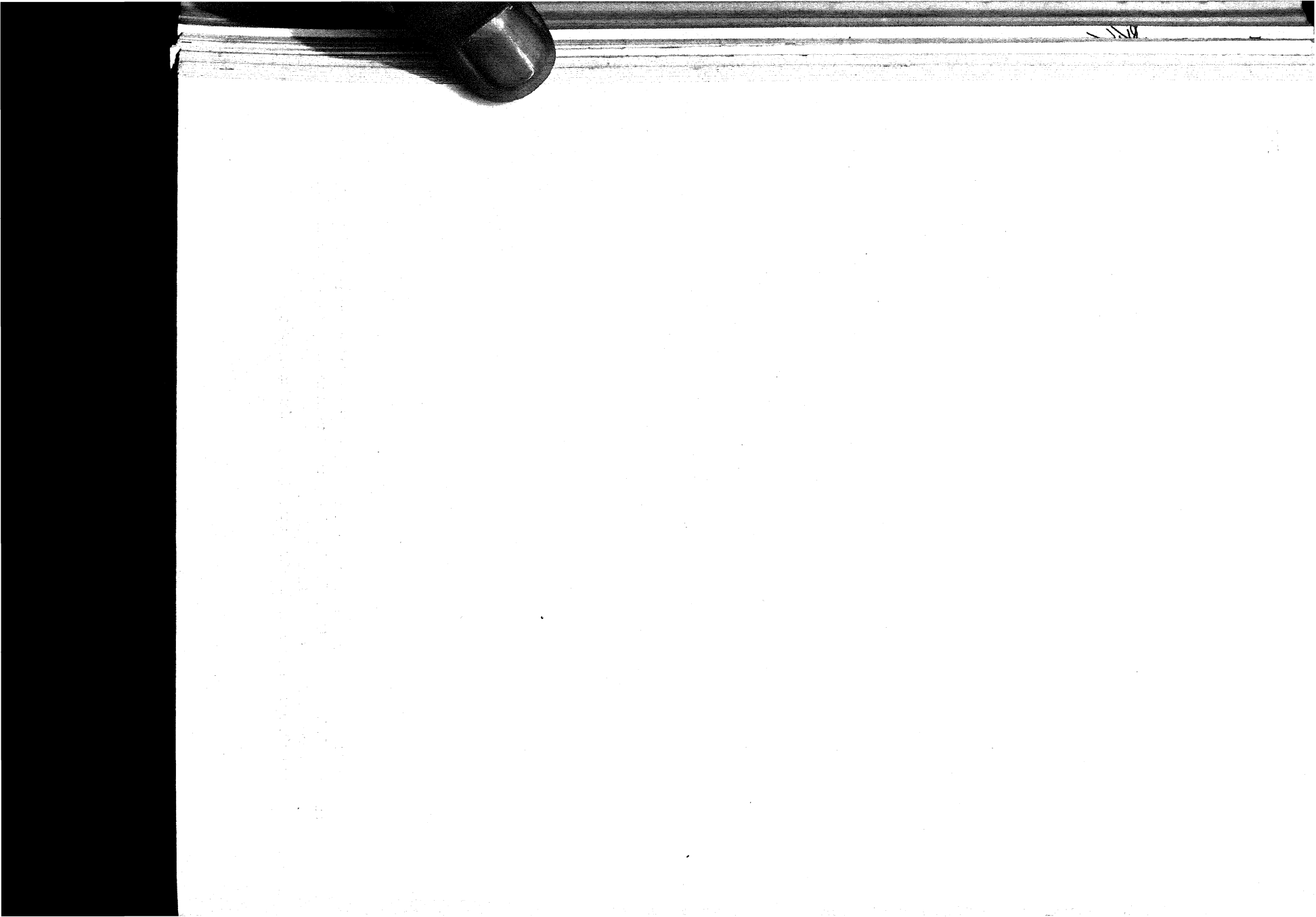


Fig. 59.

Fig. 56. Outline drawing of the section of an ovule of *Cajanus indicus* Spreng. Fig. 57. The same of an ovule of *Lathyrus sativus* Linn. Fig. 58. The same of an ovule of *Pisum sativum* Linn. Fig. 59. The same of an ovule of *Dolichos Lablab* Linn. (×200).

The position of the inner and outer integuments is to be noticed in all cases.



3. The archesporial cell divides into a primary parietal cell and a megaspore mother cell in *Lathyrus* but in the other species no parietal cell has been observed, the archesporial cell directly functioning as the megaspore mother cell.

4. As a result of two divisions, the megaspore mother cell produces a tetrad of megaspores.

5. In all the species the innermost (chalazai) megaspore functions as the embryosac mother cell; the other three degenerate.

6. As a result of the activity of the functioning megaspore a typical eight-nucleate embryosac is formed.

7. In the mature embryosac three of the eight nuclei organise the egg apparatus; the synergids are more or less beaked in all the species; only those of *Cajanus* possess well defined filiform apparatus.

8. Three nuclei at the chalazal end of the embryosac differentiate as the antipodal cells.

9. The polar nuclei migrate towards each other, fuse to form the primary endosperm nucleus in *Pachyrhizus* and *Cajanus*. In other species the polars lie side by side or one above the other.

10. The embryosac absorbs considerably the surrounding nucellar cells in all the species.

11. Sterility of ovules is of very rare occurrence under natural conditions.

12. The haploid number of chromosomes in *Pisum* and *Lathyrus* is seven and in *Cajanus* and *Pachyrhizus* is eleven.

In conclusion I wish to express my gratitude for help and encouragement in various ways to Mr. Ilabonto Banerji, University Lecturer in Botany, under whose direction this work was carried out.

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STATISTICAL NOTES FOR AGRICULTURAL WORKERS.

NO. 14.—THE USE OF RANDOM SAMPLING NUMBERS IN AGRICULTURAL EXPERIMENTS.

BY

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(Received for publication on 28th June 1933)

(With three text-figures)

We often receive enquiries regarding the most convenient method of collecting random samples or randomizing the lay-out of plots in field trials. The old method of drawing tickets from a bag or urn can, of course, be always used. But the process is extremely laborious, and in practice it is almost never possible to shuffle the tickets adequately between successive draws. In order to get over the difficulty of random sampling, Prof. Karl Pearson suggested some time ago to Mr. L. H. C. Tippett that the system of tickets might be replaced by a random system of numbers. Over 10,000 sets of 4 random numbers arranged by Mr. Tippett in 26 pages was published in 1927 (Tracts for Computers No. XV, Cambridge University Press). It contains a valuable introduction by Prof. Pearson and is practically indispensable in a statistical laboratory. But as all agricultural field workers do not have access to this tract, I am giving here a short list of 2,000 random numbers arranged in 500 sets of 4, which were obtained by taking random samples from Tippett's numbers and then re-arranging (*i.e.*, shuffling) them again in a random manner. The following examples will indicate some of the ways in which these random numbers may be used in agricultural experiments.

Example 1.—Let us suppose there are 100 plants arranged in 10 rows and 10 columns in a particular field experiment as shown in Fig. 1. It is desired to select a random sample of five plants.

—	1	2	3	4	5	6	7	8	9	0
1	*	*	*	*	*	*	*	*	*	*
2	*	*	*	*	*	*	*	*	*	*
3	*	*	*	*	*	*	*	*	*	*
4	*	*	*	*	*	*	*	*	*	*
5	*	*	*	*	*	*	*	*	*	*
6	*	*	*	*	*	*	*	*	*	*
7	*	*	*	*	*	*	*	*	*	*
8	*	*	*	*	*	*	*	*	*	*
9	*	*	*	*	*	*	*	*	*	*
0	*	*	*	*	*	*	*	*	*	*

Fig. 1.

We can identify any particular plant by the number of the row and the number of the column in which it occurs. Thus the numbers (1,1) will represent the plant in the 1st row and the 1st column; (1,9) the plant in the 1st row and 9th column; (5,4) the plant in the 5th row and the 4th column; and so on. We can conveniently settle that (0,0) will represent the plant in the 10th row and 10th column.

It is clear that any set of two figures will represent one particular plant. Instead of using two separate figures, we can also use a single number of two figures provided we adopt a convention that the first figure will represent the row and the second figure will represent the column (or *vice versa*). Any random number of two figures will then represent a plant chosen in a random manner.

In Block 1 of Table I we have, for example, the following random numbers:—

2082; 1494; 7012; 0095; 6866.

We can choose 5 numbers of 2 figures each in any way we like:—vertically downwards, from the first two columns: 21, 70, 60, 40, 08; or from the last two columns: 89, 19, 62, 42, 56; or from the 2nd and 3rd columns: 04, 80, 88, 91, 96; horizontally from first three rows: 20, 82, 14, 94, 70; from the 2nd, 3rd and the 4th rows: 14, 94, 70, 12, 00; from the 1st, 3rd and the 5th rows: 20, 82, 70, 12, 68, etc. Now each number of two figures will represent a single plant, and hence any set of 5 random numbers (of two figures each) will give us a set of 5 plants selected at random, in other words a random sample of 5 plants.

It is obvious that we can easily extend the same method to give us random samples of 6, 8, 10, 20 or any number of plants.

Example 2.—Suppose instead of 100 plants we have 10,000 plants arranged in 100 rows and 100 columns. We can now use a number of two figures to represent the number of the row, and another number of two figures to represent the number of the column. Thus the plant in the 42nd row and 37th column will be labelled by the two numbers 42 and 37; or the plant in the 3rd row and 15th column by the two numbers 3 and 15 (which may be more conveniently written as 03 and 15); the plant in the 4th row and 6th column by the numbers 04, 06; and so on, with the convention that (00,00) will represent the plant in the 100th row and 100th column. It is clear that any number of 4 figures will now suffice for identifying a particular plant, and hence any random number of 4 figures will give a plant selected at random.

Example 3.—If we have, say, 1,000 plants arranged in 10 rows and 100 columns, we can obviously use a number of 3 figures to represent a particular plant with the convention that the first figure will give the number of the row, and the last two figures the number of the column, while (000) will represent the plant in the 10th row and 100th column.

Example 4.—Suppose 1,000 plants are arranged in 50 rows and 20 columns. A number of 4 figures will again identify a plant. For example 4316 will represent the plant in the 43rd row and the 16th column. But it is clear that the number representing the row, that is the number given by the first two figures will never exceed 50; and similarly the number representing the column given by the last two figures will never exceed 20. Thus although each plant will have a particular number of 4 figures, all numbers of 4 figures will not represent particular plant. Any number greater than 5020 or any number like 4334 will not represent anything.

A slight modification in our procedure is now necessary. We can, of course, ignore all numbers which do not represent plants. But this will involve the rejection of many numbers. A better method will be to assign more than one number to each plant. For example, in this particular case we can decide that 01 and 51 will both represent the 1st row, 02 and 52 the 2nd row, 11 and 61 the 11th row, 24 and 74 the 24th row, 49 and 99 the 49th row, and 50 and 00 the 50th row. Similarly we can settle that 01, 21, 41, 61 and 81 will all represent the 1st column; 02, 22, 42, 62 and 82 the 2nd column; 11, 31, 51, 71 and 91 the 11th column; and 20, 40, 60, 80 and 00 the 20th column. There will not be any empty numbers left, so that each number will identify one particular plant (although each plant will have more than one number assigned to it). We can now use any set of random numbers of 4 figures each to represent a random sample of plants.

Example 5.—Further modifications will be necessary when the number of rows or columns is not a multiple of 10. Suppose we have 17 rows. We can now assign 5 numbers to each row. For example 1, 18, 35, 52 and 69 will all represent the 1st row; 17, 34, 51, 68, and 85, the 17th row, the rule being that only the remainder after division by 17 is to be taken into consideration (a zero remainder standing for the 17th row). But this will leave the 15 numbers 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 00 as blanks. We can, however, still continue to divide by 17 and use the remainder as before. Provided this is done, each number of 2 figures will again represent unambiguously one particular row, and we shall be in a position to use random numbers of 2 figures to specify sample rows selected in a random manner.*

* A slight inequality will however be introduced in this case. Rows 1 to 15 will each be represented by six numbers, while rows 16 and 17 will be represented by 5 numbers. The chance of occurrence of rows 16 and 17 will, therefore, be slightly less in the long run. We can better equalise the chance if we use 3 figures (*i.e.* numbers 1—999 and 000) instead of only two figures. Dividing 1,000 by 17 we get 58 as dividend and 14 as remainder. It is clear that each of the rows 1—14 will be represented by 59 figures while rows 15, 16 and 17 will have only 58 figures each. The inequality is now reduced to 1 in 58, while with two figures the inequality was 1 in 5. If we use 4 figures the chances will be still better equalised. For agricultural experiments (in which the size of samples is usually small) such refinements will not, however, be usually required. The difficulty may be avoided by rejecting all numbers above 85, so that all the rows will be represented by five numbers.

Example 6.—It is required to distribute 7 varieties (A, B, C, D, E, F, and G) to 7 plots within a "block" in a field experiment. Let us assign the numbers 1, 2, 3, 4, 5, 6 and 0 to the 7 varieties, *i.e.*, settle that the number 1 will represent A, *i.e.*, 1=A, 2=B, 3=C, 4=D, 5=E, 6=F, and 0=G. Let us use random numbers of 3 figures, and adopt the convention that only the remainder is to be taken into consideration after division by 7. Consider any particular number of 3 figures, say 725; dividing by 7 we obtain a remainder of 4; this will then represent variety D.

We can now draw 2 random numbers of 3 figures each from our plates, say 569 and 411. Dividing by 7 we obtain remainders 2 and 4. We may, therefore, proceed to allot 2=B to plot No. 4.

Plots	1	2	3	4	5	6	7
Random No.	(334)	(073)	(636)	(569)		(451)	(932)
Varieties	5=E	3=C	6=F	2=B	G	4=D	1=A

Fig. 2.

We next draw two other random numbers, say 342 and 451 which give remainders 6 and 4. We can use 4=D for plot No. 6 (because the order in which the two remainders are used is obviously immaterial). The next two random numbers are 334 and 316 with remainders 5 and 1; we can, therefore, assign 5=E to plot No. 1.

The next 2 numbers are 068, 690 with remainders 5 and 4. As we have already used both the varieties 4=D and 5=E, we ignore this set. We draw a fresh set of two numbers 073, 275 with remainders 3 and 2. We can allot variety 3=C to plot No. 2. The next 2 numbers are 014, 932 with remainders 0, 1; which enable us to assign variety No. 1=A to plot No. 7 (which will be represented by the number 0).

We have next 636, 948 with remainders 6, 3; we assign variety No. 6=F to plot No. 3. This leaves us variety G for plot No. 5.

The plots are now effectively randomized. It will be noticed that the whole procedure is extremely simple and quick; it only requires the use of a set of random numbers.

Example 7.—It is desired to select a random sample of plants from an experimental plot. The plants were not arranged in rows and columns, or in any regular manner, but were sown in a haphazard way.

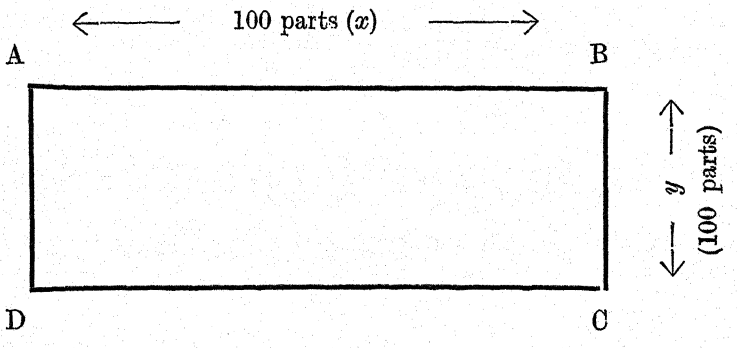

Fig. 3.

Let A, B, C, D, be the four corner points of the plot (which may be square or rectangular in shape). We can divide all the sides AB, BC, CD and DA into 100 segments, and draw (on a plan) lines through each segment parallel to the sides. The whole plot will then be divided into $100 \times 100 = 10{,}000$ small cells (each of which will be of the same shape as the plot itself). We can consider the segments along AD (or BC) to give the successive rows, and the segments along AB (or CD) the successive columns. Each small cell can then be labelled by the number of the row (or segment along AD or BC) and the number of the column (or segment along AB or CD) in which it occurs.

Thus, as in Example 1, each small cell will be represented by a number of 4 figures; and since there are $100 \times 100 = 10{,}000$ cells, each number of 4 figures will represent a particular cell. We can, therefore, proceed to use random numbers of 4 figures each to give random selections of cells. The corresponding plants lying within these random cells will obviously furnish a random selection of plants.

If the length of one side is considerably greater than the other side, we can divide the longer side into 1,000 parts and the shorter side into 100 parts. We shall have then to use 3 figures to represent segments for the longer side, and 2 figures for the shorter side, that is numbers of 5 figures each to represent particular cells.

It will be noticed that the coarseness or fineness of the division is entirely at our choice. With $10 \times 10 = 100$ cells, we require only 2 figures; but the

division is very coarse. But if necessary we can use $10 \times 100 = 1{,}000$ cells; or $100 \times 100 = 10{,}000$ cells; or $1{,}000 \times 1{,}000 = 1{,}000{,}000$ cells; and so on to any desired degree of fineness. It will be noticed that in the limit this process will reduce to using coordinates (x, y) to specify a point in the plot, where x will denote the distance from one end of the field AD, measured along say the side AB, and y the distance measured along the side AD from the end AB, the length of each side being taken equal to 10, or 100, or 1,000, or some other convenient multiple of 10.

These are only some of the uses to which random numbers may be put. Other examples will easily occur to field workers. Prof. Pearson's foreword to Tippett's tract should be consulted by every one having access to the tract. The random numbers may, of course, be taken backwards or diagonally or in any other way. The same set of numbers taken in different ways can thus furnish many more sets. One word of caution, however, is necessary. It is true that from the same set of numbers we can obtain a large number or combinations by taking the individual numbers in different ways, but we should not use the same set over and over again beyond a certain limit. In Example 1, we have altogether $5 \times 4 = 20$ random figures in Block 1, Table I. Now each plant requires 2 figures to specify its position. The set of 20 figures in Block 1, Table I, will therefore yield at the most 10 (since $20/2 = 10$) independent random plants. If we try to draw more than 10 samples from the same set of numbers (say from Block 1, Table I), it is clear that some of the numbers will no longer remain independent as some of the figures will occur over and over again and some bias will be thus introduced. The general rule is that the total number of random samples must not exceed the total number of sets of figures available. Provided this condition is not violated it is, of course, immaterial how the particular numbers are selected.

TABLE I.

2082	5606	2688	4629	3551
1494	5749	2339	8307	9238
7012	3114	1447	9732	0432
0095	3314	3419	5627	8113
6866	2145	0382	9555	8768
7286	1643	4390	5507	1407
2218	5373	0150	3655	8588
8774	6808	6639	2857	2889
0240	6061	3841	8302	9957
8793	6401	9600	6442	0172

TABLE I—*contd.*

9996	7519	8940	4275	0187
5901	6804	8329	6822	3275
1490	4995	6587	5403	1121
6887	3226	7515	0334	9889
4876	7103	2298	4117	7621
3392	3987	9739	2667	2732
0476	1250	0513	7847	6960
3256	0224	6925	3911	2118
6747	1073	0905	1945	4238
2330	4853	0647	3362	6904
7565	0328	3044	1912	1501
3561	7624	7741	4569	2655
3004	9895	6380	8898	4694
2103	0797	4079	8557	3068
8161	1106	4885	4649	4500

TABLE II.

0845	1501	7041	8726	8884
1382	2736	3981	9230	1195
6956	5478	5575	9360	6447
6037	5636	0310	0359	6595
0589	4927	1730	4497	8995
2102	2390	9498	3343	4428
3561	4996	8820	0496	5865
5397	2341	0297	5356	3074
7691	1365	3090	2427	7839
7872	7937	5748	0962	1434
8495	5160	9628	9465	3209
2096	4152	6965	6152	4258
1465	2245	0818	3836	3275
1493	0809	3941	1760	1693
2837	2642	2454	3508	0772
3830	1927	8163	0331	7553
9157	5933	7759	6911	8729
1079	0032	9151	1006	6038
1497	0782	2798	2649	8135
1175	7452	3581	4532	2850
0658	6910	4432	0136	6436
0668	7398	8869	4097	3844
2052	4564	0577	7022	4994
7052	9129	7152	3788	3993
3690	7895	5722	2031	5215

TABLE III.

9607	8606	2182	9039	9403
2130	2445	9289	3350	2317
4764	2839	1687	9803	3551
2557	7875	5816	6867	6090
7276	9687	1698	4807	7534
8986	4736	1074	8623	5911
1306	4733	5438	5208	3817
0674	1429	1161	9063	6104
5315	8416	3880	2542	5267
9917	9917	8756	2896	9215
2902	7844	5663	5943	6897
3641	0367	9614	4905	4874
5334	9693	4072	6084	4461
7149	0056	9681	3795	1840
1338	2004	7031	2548	4930
4130	1299	9253	1904	2169
5291	8839	4594	4246	8382
2580	8098	6867	1666	5813
2159	5827	5748	7605	8800
0386	3421	5866	3828	1400
0608	7866	1459	7561	9246
1498	3359	2707	2446	8798
7930	4024	9284	1058	7765
4105	4261	9978	1407	2538
8849	0557	1834	2450	1929

TABLE IV.

3939	7628	2056	3852	4277
1658	4695	5218	8680	5970
7201	9589	9408	2626	3254
7261	4891	5967	1457	5635
2270	7538	6340	8247	2924
9862	1627	8774	3008	7778
7262	7040	2726	8520	6217
5421	5619	5601	9904	1571
0572	3791	1652	2355	6299
5021	4593	2307	1359	1592
8078	4293	0315	1375	1275
3256	0262	6259	7778	4374
9004	5223	9682	8131	0909
9334	1992	8646	7431	1581
7639	7842	4939	3601	0771
8419	8457	5751	3389	5751
4920	5006	1541	5122	1541
5699	1717	3666	5655	3666
9310	9079	9821	7705	9821
6564	6467	5851	8065	5855
0523	1698	5996	4326	7217
8253	5667	9590	0778	2422
6095	3442	1173	9072	8365
1807	9825	7566	9676	9153
1920	0481	9517	1276	4466

INHERITANCE OF POLLEN COLOUR IN ASIATIC COTTONS.

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(With Plate XCII)

Introduction.

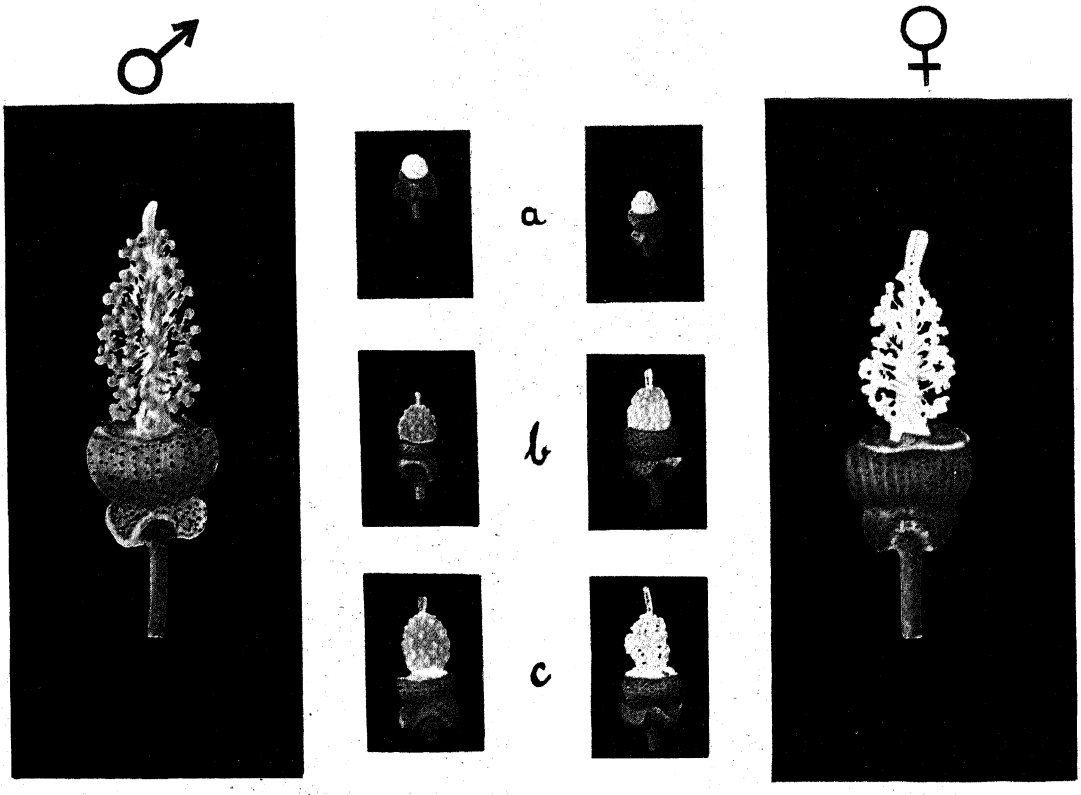
All the existent literature [Balls, 1910, 1912; Mclendon, 1912; Kearney, 1923; Bannerji, 1929; Carver, 1929; Harland, 1929, 1932] on the inheritance of pollen colour in cotton relates to the behaviour of the New World types alone. The only mention about this character in the Old World group is that made by Harland when he states that "In Old World cottons deep yellow is the usual colour though paler shades have been noticed". Since all the Asiatic types possess only half the number of choromosomes of the American cottons, it will be interesting to examine whether the mode of segregation of pollen colour in the former, will be similar to that reported in the latter.

Material and methods.

During the course of selection work in the 'Coconadas' cotton, the junior author detected a plant with cream pollen, corresponding to class zero of Harland's grading, which is a rare feature in the indigenous types. It was found to breed true in the succeeding generations and was used as one of the parents—strain 45—in the present study.

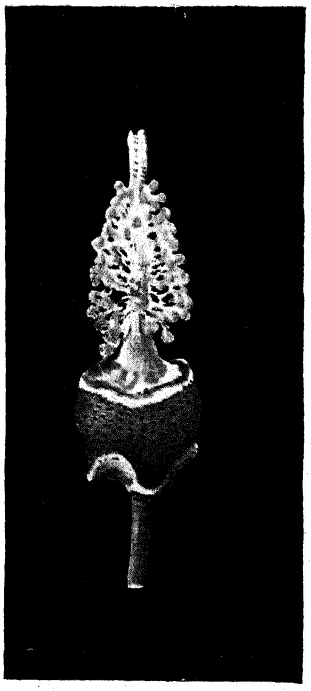
Strain 45 (grade 0) was crossed with strain 171 (grade 2·5) of Coconadas (*G. obtusifolium*) and with strain 2113 of Uppam (*G. herbaceum*) cotton. A few of the F_1's were back-crossed with the recessive parent. The F_2 populations resulting from these hybrids were classified according to Harland's grades four hours after flower opening. This study was further followed up in the F_3 generation with a view to decide definitely about their behaviour.

It may be stated here that an examination of pollen colour—usually done in the afternoons—in all the Asiatic cottons grown on the Cotton Breeding Station, Coimbatore, revealed that all belonged to grade 2·5 except *G. sanguineum*, which was of grade 3·0. It was also noted that the anthers had a darker tint in the bud

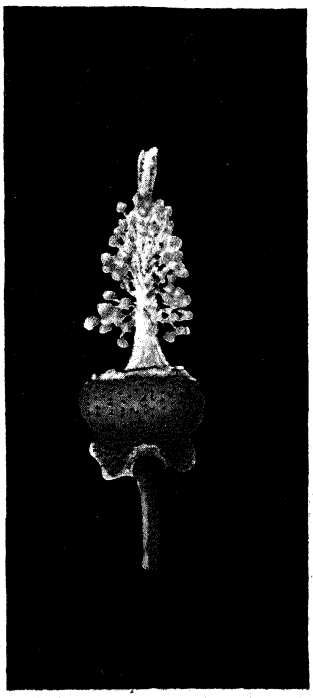


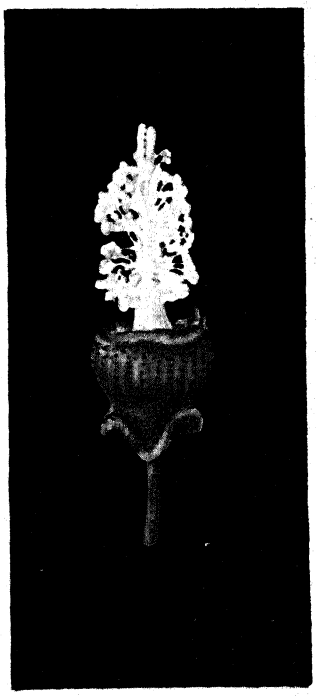
PARENTS.

a. Very young flower buds.
b. About fourteen days' old flower buds.
c. Flower buds two days prior to opening.



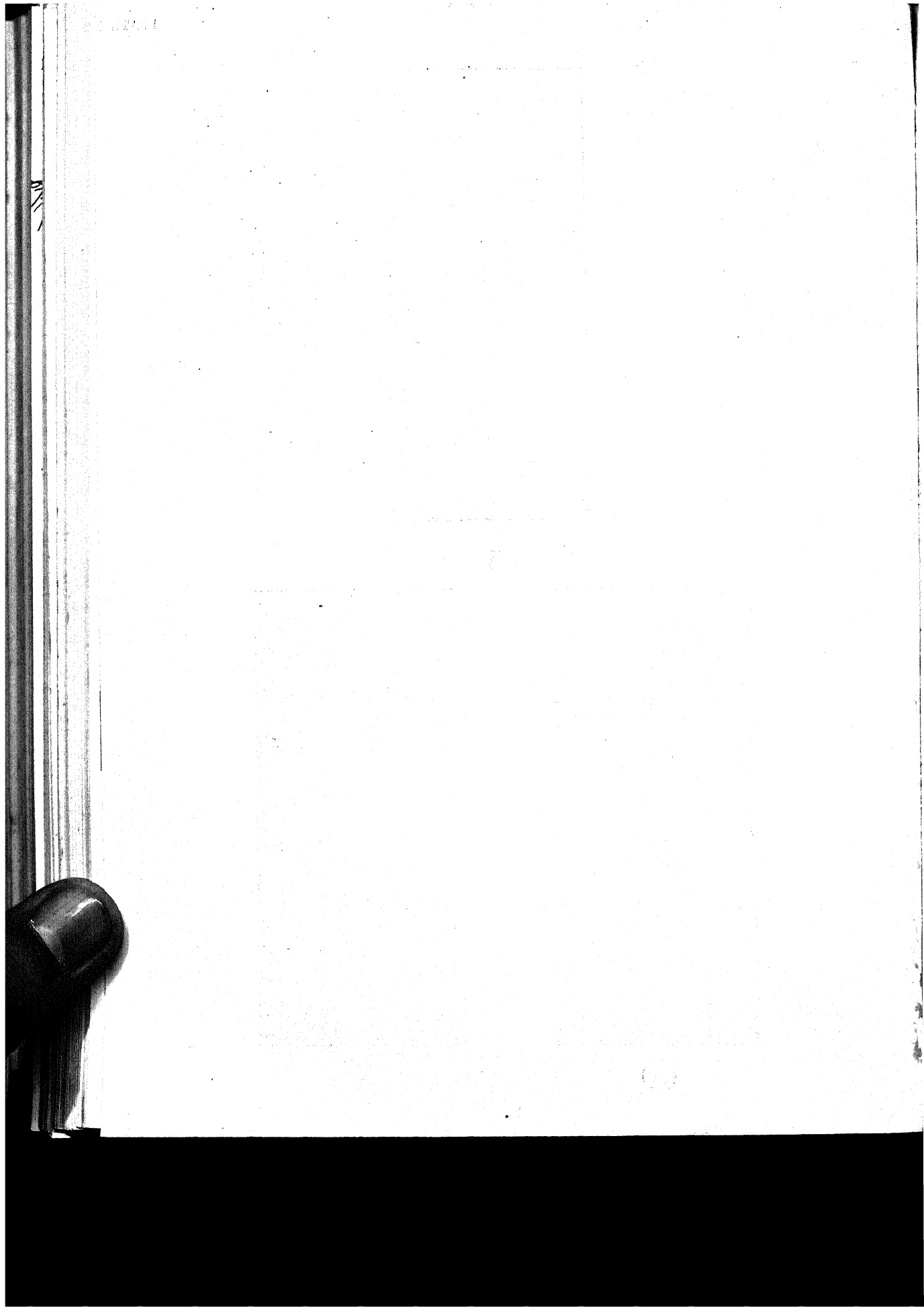
F1





③ ①

F2.



stage than on the day of flower opening (Plate XCII). Some difficulty was experienced in classification as all the flowers of a single plant were not exactly of the same shade of colour (2·5). A few of them exhibited a lower grade (2·0). Even in the same flower the pollen grains at the top were lighter than at the base of the staminal column. The anther sac too varied from pale yellow to pinkish yellow, so that minor colour differences were induced by the variation in the colour of the background. Other factors, like quantity of pollen, time of dehiscence of anthers, and presence of sterile or semi-sterile anthers, also influenced the classification. Invariably the lower grade was due to one or more of the above causes and in all such cases these had to be changed subsequently on checking to the next higher grade of 2·5.

Results.

Table I gives the data obtained in the intervarietal cross.

Table I.

Results of intervarietal cross.

Family	No. of plants with pollen grade		Remarks
	0	2·5	
45 (*G. obtusifolium*) . . .	*	..	..
171 Ditto . . .	..	*	..
F_1	..	*	..
F_2 2	27	62	..
3	25	84	..
4	93	300	..
5	12	48	..
6	42	92	..
7	17	60	..
8	21	57	..
Total .	237	703	..
Expected (1: 3) . . .	235	705	χ = 0·0227 (not significant)
Back cross 1 (171 × 45) F1 × 45 ♂ .	48	48	..
2 45 × (171 × 45) F1 ♂ .	95	85	..
Total .	143	133	..
Expected	138	138	χ^2 = 0·3623 (not significant)

It is evident that grade 2·5 is completely dominant in the F_1, and that there is a sharp segregation of 3 : 1 in the F_2. The figures obtained from the back crosses confirm the hypothesis, that only one pair of factors is concerned in the production of pollen colour in the strains under investigation. It may be noted here that the deficiency in the number of plants with yellow pollen reported by Harland in the back crosses, is perceptible only in one family.

When the results of the interspecific crosses are scrutinized (Table II) a monohybrid ratio is clearly indicated.

TABLE II.

Results of interspecific cross.

Family	No. of plants with pollen grade		Remarks
	0	2·5	
45 (*G. obtusifolium*) . . .	*	..	..
2113 (*G. herbaceum*) . . .	..	*	..
F_1	..	*	..
F_2	89	278	..
Expected (1 : 3)	92	275	$\chi^2 = 0{\cdot}1305$ (not significant)

The behaviour of the F_3 families in both intervarietal and interspecific crosses (Tables III and IV) was also in complete harmony with expectations.

TABLE III.

Results of intervarietal cross. 45×171—F_3.

Family numbers and their grades		No. of plants with pollen grade	
		0	2·5
Grade 0 . . .	1741, 1744, 1745, 1746, 1748, 1752, 1757, 1763, 1766, 1768, 1774. 1781, 1783, 1784, 1786, 1787, 1788, 1789, 1796, 1797	586	..
Grade 2·5 . .	1739, 1740, 1747, 1749, 1771, 1772, 1773, 1775, 1776, 1777, 1779, 1792, 1793, 1794	..	434
Grade 2·5 . . .	1742	13	40
	1743	3	9

TABLE III—*contd.*

Family numbers and their grades		No. of plants with pollen grade	
		0	2·5
Grade 2·5—contd.	1750	15	39
	1751	10	27
	1753	5	16
	1754	8	26
	1755	8	21
	1756	7	29
	1758	10	30
	1759	6	15
	1760	10	23
	1761	8	20
	1762	7	20
	1764	6	19
	1765	9	25
	1767	7	23
	1769	8	25
	1770	12	27
	1778	7	17
	1780	6	17
	1782	9	26
	1785	6	22
	1790	8	26
	1791	6	20
	1795	12	31
	1798	7	17
	Total	213	610
	Expected (1 : 3)	206	617 χ^2=0·3173 (not significant)

TABLE IV.

Results of interspecific cross 45×2113—F_3.

Family numbers and their grades		No. of plants with pollen grade	
		0	2·5
Grade 0 . . .	5179, 5184, 5185, 5189, 5201, 5202, 5208, 5214, 5215, 5217	166	...
Grade 2·5 . . .	5186, 5192, 5198, 5207, 5209, 5216, 5220 .	...	133
Grade 2·5 . . .	5180	10	28
	5181	9	21
	5182	9	23
	5183	8	30
	5187	8	19
	5188	7	19
	5190	2	5
	5191	6	21
	5193	7	24
	5194	10	24
	5195	8	15
	5196	5	20
	5197	7	25
	5200	7	29
	5203	5	25
	5204	9	23
	5205	4	20
	5206	5	23
	5210	2	7
	5211	7	20
	5212	3	7
	5213	8	24
	5218	3	7
	5219	9	20
	Total .	158	479
	Expected (1 : 3) .	159	478
		$\chi^2 = 0{\cdot}0084$ (not significant)	

Zero grades of the second generation invariably bred true, while in the dominant grade, some plants showed segregation, and a few bred pure for grade 2·5.

In addition to these, crosses between different species exhibiting grade 2·5, were made and their descendants were studied in detail. It was interesting to observe neither intensification nor dilution of the parental grade (Table V).

TABLE V.

Results of interspecific cross.

Family	Number of plants with pollen grade		Remarks
	0	2·5	
171 (*G. obtusifolium*)	..	*	
2113 (*G. herbaceum*)	..	*	
F_1	..	*	
F_2	..	202	
171 (*G. obtusifolium*)	..	*	
H. 1 (*G. herbaceum*)	..	*	
F_1	..	*	
F_2	..	201	
171 (*G. obtusifolium*)	..	*	
Garo Hill Cotton (*G. cernuum*)	..	*	
F_1	..	*	
F_2	..	51	
546 (*G. indicum*)	..	*	
2113 (*G. herbaceum*)	..	*	
F_1	..	*	
F_2	..	153	
43 (*G. obtusifolium*)	..	*	
546 (*G. indicum*)	..	*	
F_1	..	*	
F_2	..	197	

TABLE V—*contd.*

Results of interspecific cross—contd.

Family	Number of plants with pollen grade		Remarks
	0	2·5	
(*G. indicum* × *G. N. roseum*) F_1	..	*	
2919 (*G. herbaceum*)	..	*	
F_1	..	*	
F_2	..	212	
(*G. indicum* × *G. herbaceum*) F_1	..	*	
(*G. N. roseum*)	..	*	
F_1	..	*	
F_2	..	320	

This clearly indicated that these strains did not carry different modifying factors for yellow pollen colour.

Work on crosses between *G. sanguineum* (grade 3·0) and *G. herbaceum* (grade 2·5) is under way. Details will be published later on, when the results are ready.

DISCUSSION.

It was seen that there were no intermediate grades in the different generations in both types of crosses, and the distinction was always sharp and clear-cut, *i.e.*, the yellow and cream pollen were of grades 2·5 and 0 only. The disturbing influence of the modifying factors found by Harland in the interspecific crosses, is absent in our present study. The absence of such intergrading colours may be due to the presence of similar modifying factors in a homozygous condition in all the species studied, and may go to strengthen the suggestion made by Harland [1928] that "there is only one species of cultivated Asiatic cotton". Ware [1932] also states that the Old World cottons are more closely related to one another and differ very little in the complements of modifying factors among themselves, than the members of the New World cottons.

The data also suggest that Selection 45 in Coconadas arose probably as a single point mutation like the one found by Harland in Sea Island or Egyptian cottons. This selection has a considerable percentage of sterile anthers. It is very likely that its rare occurrence is partly due to its poor productive capacity. Measure-

ments of the sizes in the yellow and cream pollens from the segregating families did not show any appreciable differences (Table VI).

TABLE VI.

Measurement of pollen grains.

Date	Number of readings	Average diameter of pollen grains in μ		Remarks
		Grade 0	Grade 2·5	
16th December 1932 . . .	49	51·3	54·8	
17th December 1932 . . .	35	53·5	56·8	
18th December 1932 . . .	42	54·1	51·7	

ACKNOWLEDGMENTS.

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SUMMARY.

1. Segregation of the colour of pollen was studied in both intervarietal and interspecific crosses in two Asiatic cottons (*G. obtusifolium* and *G. herbaceum*).

2. It was found that the segregation was sharp and clear-cut. Only one factor is involved in the expression of yellow colour.

3. The presence of different modifying factors was not noticed even in interspecific crosses.

4. Crosses involving different species having pollen of grade 2·5 did not show either intensification or dilution.

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THE INHERITANCE OF 'LINTLESS' IN ASIATIC COTTONS.*

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Introduction.

Studies of the inheritance of lint in cotton are of considerable interest both from the practical and theoretical point of view, since they throw light on the genetic constitution of a character which gives the plant its commercial importance, and which is absent from, or poorly developed in, the primitive wild species of the genus.

Previous work on the subject has been summarised, and discussed by Harland [1932] in "The Genetics of Gossypium" and will not be dealt with here.

* The experimental work on the two lintless strains of *mollisoni* here reported, was carried out by M. Afzal, at the Cotton Research Laboratory, Lyallpur, Punjab, India, and the work on the Dharwar strain, by J. B. Hutchinson, at the Cotton Research Station, Trinidad, B. W. 1.

J. B. Hutchinson is responsible for the genetic analysis of the results.

Materials.

Three lintless strains were used in the experiments here reported.

1. Hairy lintless.—A lintless plant was discovered in 1927 in a field of *mollisoni* cotton (*G. indicum* var. *mollisoni* of Gammie, a form of *G. arboreum* var. *Nanking* according to the classification included in Harland's [1932] "Genetics of Gossypium"), near Renala in the Montgomery district of the Punjab. The plant was indistinguishable from other *mollisoni* plants in general appearance. It was fairly thickly covered with hairs, both simple and stellate, on stem, petioles and leaves, like normal *mollisoni* plants. The seeds were thickly covered with short grey fuzz, but lint was entirely absent.

2. Glabrous lintless.—Glabrous lintless plants are entirely devoid of hair throughout the plant body, and the seeds are naked save for a few short hairs, either scattered over the surface, or forming a small tuft at the chalazal end. Glabrous lintless plants have blunt buds, with the immature petals incompletely folded, exposing the tip of the stigma for some days before flowering. This character has been described by Afzal and Singh [1932] and has been studied in detail by Hutchinson (in press). Hutchinson's data show that blunt bud is associated with a short petal.

Two glabrous lintless strains of different origin were used.

Mollisoni G. L.—This strain originated as a single plant discovered in a field of *mollisoni* cotton near Sangla in the Shiekhupura district of the Punjab.

Dharwar G. L.—The origin of this strain is given by Kottur [1927] from whom seed was obtained. It arose by mutation from a Burmese strain of *G. Nanking* (*G. arboreum* var. *Nanking* according to the classification included in Harland's [1932] paper).

Experiments.

1. Inheritance of hairy lintless.

Progenies were grown from selfed seed of hairy lintless plants for five generations, and no homozygous lintless strains were extracted. Results are available from 13 families, which gave in all :—

—	Lintless	Linted	Total
Observed	118	68	186
Expected (2 : 1)	124·0	62·0	186

or a very close approximation to the 2 : 1 ratio typical of segregation for a "dominant" lethal. One family of 12 plants was obtained in which there were no linted plants. Selfed seed was obtained, and all the three plants tested gave both linted and lintless in the next generation. In this family there were in all :—

—	Lintless	Linted	Total
Parent (Observed)	12	0	12
Expected (2 : 1)	8	4	12
Offspring (Observed)	23	11	34
(3 families) Expected (2 : 1).	22·67	11·33	34·00

Deducting these four families from the totals given above leaves 9 families with

—	Lintless	Linted	Total
Observed	83	57	140
Expected (2 : 1)	93·33	46·67	140·00

$\chi^2=3{\cdot}42$, $n=1$, $P=0{\cdot}06$.

The chance of obtaining a deviation from 2 : 1 as large, or larger on random sampling is only 0·06. Taken alone, such a deviation may be regarded as of doubtful significance, but taken in conjunction with other data given below, there is no doubt that the deviation is real, and indicates that the hairy lintless type is in some families at a disadvantage compared with the linted segregates from it. Selection of a family with a high proportion of lintless plants gave a strain in which the survival rate of lintless segregates was as great as that of linted segregates.

Since the genetic evidence pointed to the action of a zygotic lethal, seeds of two sister plants, one normal linted, and the other hairy lintless, were cut open and the embryos examined. Among the seeds of the normal plant only occasional damaged embryos occurred. Among the seeds of the hairy lintless plant, about 25 per cent. contained embryos with a blackened decayed area on the dorsal surface, which usually extended far enough in to damage the plumule.

Linted segregates from hairy lintless parents bred true, and were in all respects similar to unrelated *mollisoni* plants.

Hairy lintless was crossed with normal *mollisoni*. If the hairy lintless gene be designated H^L, the cross is :—

Hairy lintless × *mollisoni*.
$H^L h^L$ × $h^L h^L$

and should give in F_1 equal numbers of $H^L h^L$ (hairy lintless) and $h^L h^L$ (normal). There were obtained :—

	Lintless	Linted	Total
Observed	30	34	64
Expected (1 : 1)	32	32	64

Linted F_1 plants bred true in F_2 and F_3.

Lintless F_1 plants segregated into hairy lintless and linted. Fifteen F_2 families forming a homogeneous group gave :—

1931	Lintless	Linted	Total
Observed	145	68	213
Expected (2 : 1)	142	71	213

or almost exactly 2 : 1.

All linted F_2 segregates tested bred true, and all lintless segregates tested again segregated in F_3. Thirty-six F_2 lintless plants gave in F_3.—

1932	Lintless	Linted	Total
Observed	321	192	513
Expected (2 : 1)	342	171	513

$\chi^2=3{\cdot}87$, $n=1$, $P=0{\cdot}05$.

The chance of obtaining such an excess of linted on random sampling is only 0·05, and the deviation must be judged significant. Low viability of the $H^L h^L$ heterozygote is again indicated.

2. *Inheritance of glabrous lintless.*

Dharwar G. L.—A strain of the Dharwar G. L. grown under number N. 19 was crossed with an unrelated form of *G. arboreum* var. *Nanking* known as N. 14. The F_1 was hairy linted, F_2s were grown and back-crossed to N. 19. Glabrous lintless behaved as a simple recessive to hairy linted, and there were in the F_2s, and back crosses :—

		Hairy linted	Glabrous lintless	Total
F_2	Observed . .	190	66	256
	Expected (3 : 1) .	192	64	256
F_1 × N. 19	Observed	110	110	220
	Expected (1 : 1) .	110	110	220

Mollisoni G. L.—A cross was made between *mollisoni* G. L. and normal *mollisoni* and gave normal hairy linted F_1.

F_2s were not grown, but families exactly equivalent are available from the cross, hairy lintless × *mollisoni* G. L. Since hairy lintless is a heterozygote two types of F_1 were obtained, hairy lintless and hairy linted (see below). The hairy linted F_1s are genetically similar to F_1s between *mollisoni* G. L. and normal *mollisoni*. Glabrous lintless behaved as a simple recessive, as in the Dharwar G. L. cross.

F_2 results are available for six families.

1931	Hairy linted	Glabrous lintless	Total
Observed	133	39	172
Expected (3 : 1) . . .	129	43	172

Thirty hairy linted F_2 plants were selfed and F_3 families grown from them. Twelve bred true to hairy linted, and 18 again segregated in a 3 : 1 ratio, giving—

1932	Hairy linted	Glabrous lintless	Total
Observed	350	96	446
Expected (3 : 1) . . .	334·5	111·5	446·0

χ^2 (3:1)=2·91. P=0·1. The deviation from the expected 3 : 1 ratio, therefore, cannot be regarded as significant.

The two glabrous lintless strains are similar in regard to the glabrous lintless character, both phenotypically, and genetically. While a direct cross between the two is needed for conclusive proof, it is at least highly probable that the same gene is involved in both cases. Subject to confirmation by results from the direct cross, the symbol h^G will be used for glabrous lintless in both strains.

3. *Hairy lintless* × *mollisoni G. L.*

On the notation suggested above this cross is:—

$$H^L\ h^L\ H^G\ H^G \times h^L\ h^L\ h^G\ h^G$$

and should give in F_1 equal numbers of hairy lintless, $H^L\ h^L\ H^G\ h^G$, and hairy linted, $h^L\ h^L\ H^G\ h^G$ plants. There were:—

—	Hairy lintless	Hairy linted	Total
Observed	28	24	52
Expected (1 : 1)	26	26	52

The behaviour of the hairy linted F_1 plants has been dealt with above. The hairy lintless plants are double heterozygotes, and should give in F_2:—

Lethal	Hairy lintless	Hairy linted	Glabrous lintless
1 $H^L H^L H^G H^G$	2 $H^L h^L H^G H^G$	1 $h^L h^L H^G H^G$	1 $h^L h^L h^G h^G$
2 $H^L H^L H^G h^G$	4 $H^L h^L H^G h^G$	2 $h^L h^L H^G H^G$	2 $H^L h^L h^G h^G$
1 $H^L H^L h^G h^G$			

The viable genotypes should occur in the proportions:—

2 Hairy lintless: 1 hairy linted: 1 glabrous lintless, giving a spurious suggestion of allelomorphism between hairy linted and glabrous lintless, with a hairy lintless heterozygote.

In eleven small F_2 families there were:—

1931	Hairy lintless	Hairy linted	Glabrous lintless	Total
Observed	60	19	10	89
Expected (2 : 1 : 1)	44·50	22·25	22·25	89·00

Fourteen F_3 families were grown from F_2 hairy lintless plants. One-third of these should split for hairy lintless only, and two-thirds should split for both types of lintless. Five families gave hairy lintless and linted only, and 9 gave all three types.

1932		Hairy lintless	Hairy linted	Glabrous lintless	Total
5 families .	Observerd . . .	58	32	..	90
	Expected (2 : 1) . .	60	30	..	90
9 families .	Observed . . .	59	41	30	130
	Expected (2 : 1 : 1) .	65·0	32·5	32·5	130·0

Agreement with expectation is good in the singly heterozygous families.

Agreement with expectation is, however, poor in both F_2 and F_3 doubly heterosygous families and in addition agreement is poor between F_2 and F_3. Evidence

has already been presented to show that the H^L gene lowers the survival rate of the plants, even in the heterozygous condition, and comparison of the 1931 F_2 results with the corresponding 1932 F_3 results given above, shows that H^L h^L heterozygotes were in defect in 1932, but not in 1931. H^L h^L heterozygotes occur among the glabrous lintless segregates in doubly heterozygous families, so the deviations from expectation in such families are best judged in terms of the proportion between linted segregates and the two kinds of lintless, rather than in terms of the proportions which the two kinds of lintless bear to the whole. Taking first the ratio of hairy lintless to linted, there were :—

Family	Year	Hairy lintless	Linted	Total
F_2	1931 . .	60	19	79
F_3	1932 . . .	59	41	100

In F_2 there was a non-significant excess of hairy lintless segregates over the expected 2 : 1 ratio, and in F_3 a corresponding defect. While neither F_2 nor F_3 differs significantly from expectation, they differ significantly from each other, χ^2 being 5·72 $n=1$ $P=0{\cdot}02$. The defect of hairy lintless in F_3 may be ascribed to the excess mortality of H^L h^L heterozygotes in 1932.

Comparing glabrous lintless with linted, there were :—

Family	Year	Linted	Glabrous lintless	Total
F_2	1931 . . .	19	10	29
F_3	1932 . . .	41	30	71

These may be regarded as samples of the same population since χ^2 is only 0·52 $n=1$ and P is about 0·5. In the two groups there were in all 60 linted : 40 glabrous lintless, a significant defect of glabrous lintless.

In F_2 and F_3 *mollisoni* families segregating for glabrous lintless, there were 483 linted : 135 glabrous lintless, a considerable deficiency of glabrous lintless, $\chi^2=3{\cdot}28$ $n=1$, $P=0{\cdot}07$. The deficiency is suggestive, but, hardly significant, and it is noteworthy that it occured chiefly in the 1932 families (F_3).

It is suggested that the deficiency of glabrous lintless in doubly heterozygous families is almost all accountable to the H^L h^L h^G h^G class, and that the seasonal difference observed in H^L h^L H^G families is masked.

Further investigation is required into the behaviour of double heterozygotes but the results obtained are in general agreement with the genetic hypotheses advanced to account for the behaviour of the two types of singly heterozygous families if allowance is made for differential viability among the extracted genotypes.

Discussion.

The three lintless strains studied are probably all recent mutants. The origin of the Dharwar strain is known. The two *mollisoni* strains originated in commercial fields but there is only one occurrence on record of each strain.

A lethal, such as hairy lintless with a heterozygote often at a disadvantage, is most unlikely to spread in a commercial crop. Contamination of a commercial strain of cotton by a deleterious recessive gene occurred in Sea Island cotton, where 'crinkled dwarf' persisted in field cultivations before the organization of an improved seed supply. The same gene is also reported to have occurred with considerable frequency as "wrinkled leaf" in certain Egyptian strains.

It is conceivable that glabrous lintless might persist to the detriment of a commercial variety, if cotton were grown continuously on the same land. Any kind of rotation would automatically eliminate it, since naked seed would never be picked, and would simply drop on the ground round the parent plant.

The lethal hairy lintless is of considerable genetic interest. It resembles the deficiencies reported from Drosophila, caused by loss of a short length of chromosome and causing a somatic abnormality when heterozygous and death when homozygous. Deficiency types are also usually of low viability when heterozygous.

Such a gene is usually termed a "dominant" lethal. There seems to be no justification whatever for the term, since the only known characteristic of the homozygote is its lethal action, in which the heterozygote differs from it. Since the heterozygote differs from the normal, it is reasonable to suppose that it is in some way intermediate, as for example, the heterozygous creeper fowl is intermediate in development between the normal and the lethal homozygote, as shown by dissection of lethal embryos [Landauer, 1932].

Summary.

(1) Two types of lintlessness are described and their behaviour demonstrated.

(2) The results obtained are interpreted as resulting from the action of two genes.

(a) $\mathbf{H^L}$, lethal in the homozygous condition, and giving rise to a hairy lintless type which is rather weak when heterozygous.

(b) $\mathbf{h^G}$, recessive to normal and giving a completely glabrous plant, with only a very few short hairs on the seed.

(3) The origin and possible influence of the two genes is discussed.

(4) The use of the term "dominant" for a lethal gene with a heterozygote differing from the normal is discussed and its inaccuracy pointed out.

ACKNOWLEDGMENTS.

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SELECTED ARTICLE

THE PRESENT POSITION AND FUTURE PROSPECTS IN RELATION TO THE BIOLOGICAL CONTROL OF PRICKLY PEAR.

BY

ALAN P. DODD.*

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The report that follows has been prepared by the Officer-in-Charge of the Commonwealth Prickly Pear Board's investigations, and has been made available by that body for publication. The Board is financed by contributions from the Council for Scientific and Industrial Research and the States of Queensland and New South Wales in the proportion of 2 : 1 : 1. At present, it is constituted as follows :—W. L. Payne (Queensland Department of Lands), (Chairman) ; G. Lightfoot (Council for Scientific and Industrial Research) ; Professor E. J. Goddard (Council for Scientific and Industrial Research) ; and G. D. Ross (New South Wales Department of Agriculture).

The work described in the report has been particularly successful, and quite an outstanding instance of the economic value of scientific research. This will be obvious when it has been seen from the report that the original pear infestation of 60 million acres—an area slightly larger than the whole State of Victoria, and also very little less than the total area of Great Britain and Northern Ireland—has all been attacked, and that with care there is every prospect of entirely ridding Australia of the pest in a comparatively short time. Science can thus fairly claim to have almost redeemed to Australia, and at a comparatively infinitesimal cost, a province of the size of the State of Victoria, and one which bade fair to become utterly useless.—Ed.

Summary.

The past three years have brought a very great change in the prickly-pear situation. Widespread destruction of the pest has followed the general establishment of *Cactoblastis*, to such an extent that the greater part of the original pear has collapsed in Queensland and the northern areas of New South Wales. In Queensland a vigorous policy of the development of pear lands for closer settlement is being pursued—a wonderful tribute to the efficiency of insect destruction.

* Officer-in-charge of the Commonwealth Prickly Pear Board's investigations.

But there is still particular need for scientific research and investigation. Re-growth, which invariably springs up after the initial collapse of prickly-pear, is a feature of the situation in many districts. Although *Cactoblastis* destroys this secondary wave of the pest readily, the Board is making a special endeavour to establish other insects for its more rapid control. Natural parasites kill a percentage of the *Cactoblastis* population; mortality from these agencies is not a serious factor and does not appear to be increasing; nevertheless, the question demands continued study. In the Hunter River districts of New South Wales, insect destruction has been much slower than elsewhere, but is now giving promise of eventual success.

The tiger-pear, *Opuntia aurantiaca*, which spreads very rapidly, is being made the subject of a special investigation for the introduction of its particular insect enemies. The tree-pear (*Opuntia tomentosa*) position in Central Queensland is being watched carefully.

In conclusion, it should be emphasized that the biological control of prickly-pear has been, up to the present, an outstanding success—a success that could hardly have been visualized five years ago.

1. Progress to May, 1929.

The last publication by the Board dealt with the progress of the biological control investigations to May, 1929. At that time, cochineal was generally established throughout the pear areas: it had considerably reduced the height and density of the pear infestation in the heavily-timbered brigalow and belar scrubs, and had brought about very effective destruction of *Opuntia stricta* in Central Queensland. The prickly-pear red spider, *Tetranychus opuntiae*, had co-operated with cochineal in the thinning out of the dense pear in the scrub areas. The plant bug, *Chelinidea tabulata*, was established in enormous numbers at many points, where it was assisting to control the fruit and new growth of the pear. The large-scale distribution of *Cactoblastis cactorum* had been commenced; around some of the centres where the earliest experimental liberations in 1926-27 of this insect had been placed, the destruction of the pest over areas of from a few to 1,000 acres indicated, in some degree, the remarkable progress that might be expected in the near future. But the greatest success had been achieved in the virtual checking of the spread of the pest, a huge increase estimated at nearly 1,000,000 acres annually, as a result of a combination of insect activities and of energetic poisoning methods adopted or enforced by the State prickly-pear organizations.

2. Progress since May, 1929.

The campaign of *Cactoblastis* distribution was carried out on a most extensive scale by co-operation between the Board and State authorities, and was practically

completed by the end of 1930, when 3,000,000,000 eggs of this insect had been released throughout the length and breadth of the entire pear area of Queensland and New South Wales, either by direct Government action or through supplies given free of cost to land-owners. So quickly did *Cactoblastis* become established that, by the end of 1931, it could be said that it existed on practically every acre of the tremendous pear infestation of both States; and so rapidly did it increase that widespread collapse of the primary pear followed its activities in every district except the more southern pear area of New South Wales.

Thus the past three years have witnessed a very sudden change in the prickly-pear situation. The success of *Cactoblastis* has been most spectacular. Over enormous areas the original dense pear, that had flourished unchecked for years, has been destroyed. This statement is not intended to convey the impression that the pest has been completely annihilated, for a secondary growth is present in greater or less degree; the re-growth question will be discussed more fully in a special section of this review. As an example of the remarkable progress achieved by *Cactoblastis*, one instance may be given. In August, 1930, the continuous and almost unbroken pear belt along the Moonie River, Southern Queensland, showed for 150 miles no destruction, and so light an infestation of *Cactoblastis* that further distribution was considered. However, the increase of the insect was so rapid that in August, 1932, two years later, 90 per cent., of the primary pear had disappeared. In Queensland, the chief remaining large belt of the two pest pears, *Opuntia inermis* and *Opuntia stricta*, is between Goondiwindi and the Moonie River. Probably 80 per cent. of Queensland's dense primary pear has been destroyed. Very fine results have been achieved in the North-west and the Pilliga State Forest areas of New South Wales; it is estimated that the primary pear has been reduced in all pear districts of that State, excepting the Hunter Valley and Camden districts, by from 50 to 60 per cent.

But as the effectiveness of *Cactoblastis* has increased, that of the other pear insects has diminished. The dense concentrations of *Chelinidea tabulata* have decreased in the past two years to rather scattered numbers. Red spider, as an effective controlling agency, no longer counts. The sphere of usefulness of cochineal has been restricted to the sporadic destruction of new growth. The favorable results at the present juncture can be attributed mainly to the work of one insect, namely, *Cactoblastis*.

3. Reclaiming of the Land.

The Queensland Government immediately took advantage of the first widespread destruction of prickly-pear to promulgate a comprehensive scheme for the development for pastoral, grazing, and agricultural purposes, of land retrieved from

the pest by insect agency. The programme is being pushed forward expeditiously. Already 1,514,881 acres of pear land have been re-selected for mixed farming operations, and 1,701,308 acres for grazing, all with development conditions. Ring-barking and falling of the useless timber, the clearing of roads and fence lines, and the erection of fences are proceeding apace. The homes of new settlers have made their appearance. Artificial grasses are being sown as the clearing of the timber progresses. Crops have already been grown successfully.

This marked evidence of progress is an outstanding tribute to the success of the biological control campaign. Within the next few years, great areas of former useless pear land will be brought into productiveness. The many new settlers will mean the growth of townships within the former prickly-pear area. Indeed, at Chinchilla, a new butter factory, shops, etc., already point to greater expansion in the near future.

The development of pear lands, except in the case of a few small areas, has been possible within the past two years only. Hence the work of bringing the reclaimed land into productiveness is as yet in its initial stages.

4. The other side of the picture.

The spectacular destruction of *Cactoblastis* has tended to give the impression that the prickly-pear problem has been completely solved, and that no further research work is necessary. When the extent of the collapse of the primary pear is realized, and when mile after mile of dead and rotting pear is viewed, the tendency to magnify the admittedly wonderful results and to overlook the incompleteness of the destruction is natural. It is therefore necessary to point out in what manner the destruction is incomplete, and to indicate the many aspects of the problem that require continued attention.

(*i*) *Re-growth.*—Care has been exercised in the foregoing sections of this article to distinguish the destruction by *Cactoblastis* as the collapse of the primary or original prickly-pear. But this destruction, spectacular as it has proved, is far from meaning the complete annihilation of the pest. Immediately after the initial collapse of the pear, one sees nothing but dead pear for a few months. The butts and roots, however, have not been completely destroyed, and when the growing season of the plant, September-December, arrives, re-growth appears. A secondary growth is not peculiar to insect destruction, for it invariably springs up after poisoning operations among dense pear.

In the early days of *Cactoblastis* progress, re-growth was not a pronounced feature. The separate areas of destruction were not extensive, and the insect population in the surrounting standing pear soon overflowed on to the new growth, and brought about its control rapidly. But when the activity of *Cactoblastis*

encompassed the collapse of the whole or major portion of the primary pear in a district, the population of the insect suddenly dropped through starvation to very low numbers, and the small residue was quite inadequate to destroy the recurring growth immediately.

The first big wave of re-growth arose in the early summer of 1930. In many areas, it was subjugated during the summer by *Cactoblastis*, which occurred in very large numbers in other portions of the same districts. However, in the Chinchilla district, the vigorous re-growth flourished unchecked throughout 1931; the *Cactoblastis* infestation, at first very light, increased in each succeeding generation, and the new growth was brought under control in the 1932 winter, or nearly two years after its appearance.

Another example of the control of re-growth may be given. In December 1931, a very dense and vigorous re-growth over several thousand acres on the eastern side of the Mungle Scrub, New South Wales, had reached the fruiting stage. The *Cactoblastis* population, which must have been light indeed eighteen months earlier, was now most satisfactory. Six weeks later, at the end of January 1932, the whole of this re-growth had collapsed.

Following the great advance of destruction of primary pear, the recurring growth of the 1931 summer involved very considerable areas. Much of this secondary wave of the pest still flourishes, fifteen months later, and in places has recently flowered and fruited. However *Cactoblastis* is present wherever re-growth occurs. This succulent type of pear is the most favorable medium for the rapid increase of the insect, and there is no reason to expect that the control of existing areas of re-growth will not be brought about within a short space of time. In Central Queensland, for some reason, possibly because of dry winter and early summer months, re-growth has not attained dense proportions and has not escaped, even if temporarily, the attention of *Cactoblastis*.

In June, 1931, the Board decided that, although the prospects of control by *Cactoblastis* were exceedingly hopeful, it would be unwise to leave the eventual control of re-growth to *Cactoblastis* alone. Hence a programme for the introduction of new strains cochineal was commenced. Supplies of these insects have been secured from several places in America, and are being reared, with a view to their distribution in the near future. Furthermore, an endeavour is being made to import from North America a particular insect, Mimorista, the caterpillars of which feed on young growth solely. With these insects co-operating with *Cactoblastis*, it is hoped that more rapid control of re-growth may be brought about.

(*ii*) *Natural enemies of Cactoblastis.*—The future of *Cactoblastis* depends upon the extent of the controlling influences exercised by disease and parasitic agencies. Disease organisms are always present among the larvae, and have at times assumed

serious epidemic proportions. However, as outbreaks are sporadic, and appear to be restricted to localities where the larvae are heavily concentrated, diseases are unlikely to bring about control of this insect.

Several native parasites have already turned their attention to *Cactoblastis*, and two have assumed some importance. The investigation of the habits and the controlling effect of these parasites is an important phase of the Board's work. Records of the degree of parasitic attack are gathered from many different localities, in order that the general position in the various districts may be gauged. At present, the control by parasites averages 15 per cent. in Central Queensland and North-west New South Wales, 5 to 10 per cent. in Southern and South-west Queensland, and 20 per cent. in the Hunter River district, New South Wales. Thus, parasites are not exercising any important degree of control. In the past two years, the percentage of mortality from parasitic attack has not increased, and there is no reason to anticipate that it will increase in the future. If *Cactoblastis* were ever to be rendered impotent, it would mostly probably be due to the controlling action of parasites. Hence it is essential that scientific observation and investigation should be maintained on this important question.

5. Other problems.

The re-growth situation, and the extent of parasitism, may be considered the main question of the future, since they are pertinent to the whole of the prickly-pear area. There are, however, various other problems of a more or less sectional nature.

(*i*) *The Hunter River situation.*—Although large-scale destruction of prickly-pear is being secured over the major portion of the infested area, there are certain districts where *Cactoblastis* and other prickly-pear insects have not given entirely the same favourable results. The largest of these sections is the Hunter River Valley, where the dense pear infestation occupies probably 2,000,000 to 3,000,000 acres. The Hunter River situation has been, for the past two years, the subject of a special investigation by the Board. It has been ascertained that the slower progress of *Cactoblastis* is due to a combination of climatic factors and of soil conditions affecting the greater portion of the pear in this area. Until 1931, the results of the extensive distribution of *Cactoblastis* had been disappointing, in that the insect had failed to become established generally. In the past eighteen months, however, *Cactoblastis* has made appreciable progress; areas of destruction somewhat limited in extent, occur at various points, while a light infestation has become fairly general throughout the district. It is hoped that this progress will continue, and that eventually the Hunter River pear will be brought under control.

(*ii*) *Tiger-pear* (*Opuntia aurantiaca*).—This plant occurs in many places in Queensland and New South Wales. Although the total infestation is not very great, possibly not more than 25,000 acres, it is increasing rapidly, the rate of spread being much greater than that of the main pest-pears. Moreover, the application of poisoning methods has not succeeded in coping with this dangerous plant, which is a very serious pest in South Africa.

Cactoblastis will destroy the upper growth, but not the underground bulb. The recuperative powers of the plant are so great that a few months after its apparent destruction it has regained its former size. When the failure of *Cactoblastis* to control this pest had been ascertained, the Board despatched two officers to South America eighteen months ago to undertake a special investigation of the insect enemies of *O. aurantiaca* and its near allies. A strain of cochineal attacking *O. aurantiaca*, has recently been received from the Argentine, where other insects are being studied, with the view to their early introduction into Australia.

(*iii*) *Tree-pears.*—Extensive areas of tree-pears, *Opuntia tomentosa* and *O. streptacantha*, more particularly the former, occur in Central Queensland. In the case of *O. streptacantha*, a special strain of cochineal from Mexico is succeeding in destroying the young plants, and is causing damage to the large plants.

As regards *O. tomentosa*, *Cactoblastis* will destroy the young plants, but will not attack the larger plants. The control of the seedling plants would seem assured while *Cactoblastis* is present on *stricta* and *inermis* in the same district, since there is always a suitable food supply for the caterpillars, and the resulting moths will deposit eggs on any young *O. tomentosa* plants that may arise. Hence, the spread of tree-pear is prevented, and the large plants must gradually die of old age. But, in the event of the *O. stricta* and *O. inermis* infestation being eradicated, the control of young tree-pear may cease, and it may become necessary to take further steps toward the introduction of particular insect enemies of this plant.

ABSTRACTS

The inheritance of virescent yellow and red plant colours in cotton.—D. T. KILLOUGH and W. R. HORLACHER. (*Genetics*, Vol. 18, No. 4, July 1933).

Deficiencies in the amount of chlorophyll in the cotton plant have been reported in relatively few instances. In all the chlorophyll deficient types so far reported, the deficient portions are yellow, due to the presence of carotinoid pigments, *viz.*, carotin and xanthophyll, and the absence of green chlorophyll. The authors have described a new type which they have called virescent yellow. The plants are greenish yellow when young, due to a partial deficiency of chlorophyll. The chlorophyll gradually increases in amount so that at maturity these plants are not readily distinguishable from normal green plants. Red-leaf cotton is produced by the distribution of anthocyanin pigment throughout the plant.

Data are presented confirming the results secured in *G. hirsutum* by McLendon, Thadani, Ware, and Carver which indicate that red leaf is a simple dominant to green. The F_1 hybrid is light red and the segregating generations show single-factor inheritance. It is however apparent from the experiments of the authors that two factors are concerned in producing these colours, one of which is common to both. Thus red may be designated as **RV** and normal green as **rV**.

A similar mode of inheritance was observed in crosses between virescent yellow and normal green which are both free from anthocyanin pigment. The F_1 hybrid was green. The virescent yellow is thus functioned by **rv** and green by **rV**.

Virescent yellow, crossed with red leaf, gave F_1 hybrid light red and the F_2 generation showed that these colours differ in two pairs of factors which are inherited independently, giving rise to two new phenotypes, *viz.*, bronze and green in addition to the parental forms, *viz.*, virescent yellow and red. Expectations were fulfilled in the F_3 generation. The genotype of the original red-leaf parent was **RRVV** and that of the virescent yellow **rrvv**. The green segregates were **rrVV** or **rrVv**. The bronze segregates were due to the action of the red-leaf gene **R** on virescent yellow and were of the genotypes **RRvv** which is dark bronze and **Rrvv** which is light bronze. All four pigments, anthocyanin, chlorophyll, xanthophyll and carotin are present in bronze plants and combine in such proportions as to produce this colour type. Mature bronze plants are practically indistinguishable from red-leaf plants. In the F_3 generation some dark red plants **RRVV** bred true and others **RRVv** split into dark red and dark bronze. All the light red plants, **RrVV** and **RrVv**, showed splitting according to expectations. The dark bronze plants, **RRvv** ,red true and the light bronze, **Rrvv**, gave dark bronze, light bronze and virescent yellow. Some of the green plants, **rrVv** split into green and virescent yellow as expected. There was no homozygous green plant, **rrVV**, in the F_3 culture. All the virescent yellows, **rrvv**, bred true.

In short bronze is a red on virescent yellow, while red is a red on green. This mode of inheritance in this cross is a case of interaction of two factors, which are independently inherited. One parent, *viz.*, red leaf combines two dominants and the other parent, *viz.*, virescent yellow, two recessives. The new segregates or the extra parental forms combine a single dominant and a single recessive. The presence of one dominant gives rise to bronze and that of the other to green. (K. I. T.).

A possible biological control of the clover springtail or lucerne flea (*Sminthurus viridis* L.) in Western Australia. H. WOMERSLEY, F.E.S., A.L.S. (*J. Council for Scientific and Industrial Research*, Vol. 6, No. 2, May 1933).

1. In certain areas of Western Australia, a species of Bdellid mite, *Biscirus lapidarius* Kramer, possibly an introduction from Europe, has made its appearance in paddocks infested with the clover springtail (lucerne flea), *Sminthurus viridis* L.

2. Field observations extending over two years have shown that this mite is an active predator on Sminthurus, and reduces the population to negligible proportions within a comparatively short time.

3. Transportation to other areas has been partially successful and the areas have been cleared of Sminthurus.

4. As the breeding up of the mite in large numbers does not seem feasible, specimens can best be transferred from localities where active attack is proceeding.

5. The species, its immature stages, and partial life-history, are described and figured. (*Author's summary*).

NOTE

THE INTERNATIONAL ORGANISATION OF CHEMICAL DOCUMENTATION.

(49, Rue des Mathurins, Paris 8e.)

Questions concerning documentation have of late assumed more and more importance. Scientific and technical documents increase on all sides in such numbers that it becomes more and more difficult to gather useful material for the benefit of inquirers. There are many bodies that deal permanently with the registering, classing and diffusion of such documents. Now the coordination of the respective activities of these institutions on an international basis has become necessary in order to permit them to carry on their work efficiently.

As regards the province of chemistry a step was taken in 1932, in the scientific and technical sphere, by the entry into activity of the international office of chemistry, created by international convention, and having its headquarters in Paris.

Its first act was the summoning of a Conference of Experts, which included the following personalities: Messrs. F. Donker Duyvis, Member of the Council of Patents, The Hague; P. Dutoit, Professor at the University of Lausanne; F. Haber, Director of the Kaiser-Wilhelm Institut für Physikalische Chemie und Electrochemie, Berlin; E. Hauser, Member of the Academy of Sciences, Madrid; Ch. Marie, Secretary General of the Comitè International des Tables Annuelles de Constantes, Paris; N. Parravano, Member of the Academy of Italy, President of the Comitato Nazionale di Chimica, Rome; G. Peny, President of the Federation of Chemical Industries of Belgium, at Brussels; J. C. Philip, Professor at the Imperial College of Science and Technology, London.

The work of this Conference of experts led to the adoption of a certain number of recommendations fixing the three principal tasks of the Office:

I.—To render accessible to all interested persons the already existing documentation, accumulated in the various centres, depots and collections.

II.—To guide the chemical documentation which is in course of production, in such a way as to facilitate its registering, filing and diffusion, by methods found to be the best.

III.—To ensure coordination between the documentation relative to chemistry and that concerning other scientific knowledge in the field of international documentation.

Thanks to these varied operations, the users of such documentation will find that all over the world a practical and rational organisation of documentation in chemistry is being carried out systematically and progressively, liable to be more and more effectively adapted to their needs.

NOTICE OF BOOK

The Sutlej Deodar : its Ecology and Timber Production. By R. Maclagan Gorrie, D.Sc. (*The Indian Forest Records, Silvicultural Series*, Vol. XVII, Part IV, 1933, pp. 140. Government of India Publication Branch, Calcutta.) Rs. 3-2-0 or 5*s.* 6*d.*

This publication is of special interest as an ecological study of one of the most important of Indian timber trees—deodar. The author's 'Introduction' and 'Summary', which are quoted below, give a brief outline of what has been attempted in correlating the timber value of this tree with the local vegetation which grows along with it.

Introduction.

The object of this paper is to trace the relationship between the plant associates of the deodar and its value as a timber tree. The deodar (*Cedrus deodara* Loudon) is the most valuable tree in the North-West Himalaya, and the Sutlej Valley forests are one of the main sources of its supply for the markets of Northern India.

The deodar occurs in a belt of forest along both sides of the Sutlej Valley stretching from the outer hills of the Lesser Himalaya to the Tibetan border, and it grows under climatic conditions varying from the heavy monsoon of the outer hills to the arid country behind the main ranges of the Himalaya, whose precipitation consists almost entirely of winter snowfall. The plants associated with the deodar vary greatly between these two extremes, and the correlation of these plants with the varying capacity of the deodar as a timber producer should serve a useful purpose in clarifying our knowledge of Himalayan sylviculture.

The identifications were made in the field with the aid of Parker's *Forest Flora for the Punjab and N. W. F. P.* [1924], which deals with trees and shrubs only, and of Collett's *Flora Simlensis* [1902], which covers only the moister areas of the outer ranges. No systematic collection of herbarium specimens was made, but many plants were referred for identification to Mr. R. N. Parker, Forest Botanist, Forest Research Institute, Dehra Dun, whose help I acknowledge most gratefully.

This paper was submitted as a thesis for the Degree of Doctor of Science of Edinburgh University in May, 1930.

Summary.

1. The habit of growth of the deodar (*Cedrus deodara* Loudon) alters entirely between the two extreme conditions of monsoon rainfall in the outer hills and

winter snowfall as the only precipitation of the Tibetan border. In the outer hills it keeps to the best drained spurs and ridges, while in the inner hills it seeks the gentler slopes and cooler aspects which retain their snow-beds longest into the spring.

2. The plant associates of the deodar alter completely between these two extremes, except for the blue pine (*Pinus excelsa* Wall.) which accompanies the deodar throughout and retreats uphill toward the snow-beds of the inner ranges in a similar manner.

3. The deodar's capacity as a timber producer alters markedly with climatic changes, and these alterations have now been correlated with the changes in itr plant associates.

4. For practical use in the field, the deodar itself is the best indication of the quality class of any existing crop, and it is proposed to employ vegetation lists only in the determination of the *site quality class*, where the existing crop is an abnormal one. The plants which indicate optimum conditions for deodar may be summarised as follows :—

Moist Zone (deodar in mixed crops with spruce and blue pine).

Adiantum capillus-veneris, Linn. and venustum, Don.
Ainsliæa apteta, DC.
Arundinaria falcata, Nees.
Asparagus filicinus, Buch.-Ham.
Fragaria vesca, Linn.
Primula denticulata, Sm.
Smilax parvifolia, Wall. and vaginata, Decne.
Spiræa bella, Sims. and vestita, Wall.
Urtica dioica, Linn.
Viola patrinii, Ging. and serpens, Wall.
Wulfenia amherstiana, Benth.

Dry and Arid Zones (deodar in pure crops).

Artemisia vestita, Wall.
Asparagus gracilis, Royle.
Astragalus chlorostachys, Lindl.
Atropa belladonna, Linn.
Bupleurum candollii, Wall. and lanceolatum, Wall
Desmodium tiliæfolium, G. Don.
Fragaria vesca, Linn.
Indigofera gerardiana, Wall.
Lilium polyphyllum, D. Don.
Philadelphus tomentosus, Wall.

Polygonatum multiflorum, All. and verticillatum, All.
Polygonum affine, D. Don. and molle, D. Don.
Thalictrum foliolosum, DC., javanicum, Blume, and minus, Linn.
Viola patrinii, Ging. and serpens, Wall.

5. In employing the vegetation lists presented in this paper, the ground flora in any given deodar crop or planting area should be studied and compared with the listed normal for the area, according to its position in the moist, dry, or arid zone, and the peculiarities of the common plants should be referred to in the analysis (Chapter VIII).

6. As the ground flora of deodar crops with a canopy consists largely of herbs, it follows that the whole of the ground cover, including herbs, ferns, and grasses as well as shrubs, should be studied for guidance in sylvicultural work.

7. Experience has shown that the drier types of deodar forest require a slower and more gradual method of regeneration than the orthodox Shelterwood System, and that marking for felling must be governed largely by the necessity for providing side shade against the hottest sun until young crops are established. A study of the component plants of the ground cover will give useful indications as to the amount to which any given crop should be opened up.

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